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CHOLINERGIC INVOLVEMENT IN PAIN INHIBITION

City University of New York PH.D. 1986

CHOLINERGIC INVOLVEMENT IN PAIN INHIBITION

by

ELLEN SPERBER FREEBERN

A dissertation submitted to the Graduate Faculty in Psychology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

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Abstract

CHOLINERGIC INVOLVEMENT IN PAIN INHIBITION

by

Ellen Sperber

Advisor: Professor Richard Bodnar

Acetylcholine and its receptor agonists increase pain thresholds and may be important in the mediation of opioid and non-opioid forms of pain inhibition. The present study assessed the effect of muscarinic receptor antagonists, scopolamine (Ø.ØØ1-1Ø.Ø mg/kg) and methylscopolamine (1.Ø-1Ø.Ø mg/kg) upon analgesia induced by cold-water swims (CWS), 2-deoxy-D-glucose (2DG) glucoprivation, forepaw (FPS) and hindpaw (HPS) footshock, pilocarpine, D-ala-D-leu-enkephalin (DADL), beta-endorphin (BEND) or morphine. Scopolamine and methylscopolamine significantly reduced CWS analgesia on the jump test 3Ø min after the swim and eliminated this effect 6Ø min following the swim. In contrast, both muscarinic receptor agonists potentiated CWS analgesia on the tail-flick test and increased CWS hypothermia effects and these were correlated. Scopolamine and methylscopolamine increased the duration of 2DG analgesia, particularly on the jump-test. However, both muscarinic receptor antagonists

reduced 2DG hyperphagia in a dose-dependent manner. This effect was correlated with the ability of scopolamine and methylscopolamine to reduce basal food intake. In contrast to their mediation of the neurohormonal analgesic responses of CWS and 2DG, neither scopolamine nor methylscopolamine altered neurally mediated FPS or HPS analgesia. Scopolamine, but not methylscopolamine, eliminated pilocarpine analgesia on the jump test and tail-flick test indicating differences in cholinergic mediation of CWS analgesia relative to this muscarinic receptor agonist. Scopolamine and methylscopolamine effects upon analgesic and other stress responses could not be attributed to shifts in baseline jump thresholds, tail-flick latencies or core body temperature changes. Despite the postulated synergy between cholinergic and opiate agonists, scopolamine potentiated DADL and morphine, but not BEND analgesia on the jump test. These results indicate that the cholinergic system mediates pain inhibition independently of the endogenous opiate system. The different patterns of reductions in CWS and pilocarpine analgesia by muscarinic antagonists suggest peripheral and central cholinergic influences. That some analgesic responses are reduced while others are potentiated by muscarinic receptor antagonism provides further evidence for heterogeneity in pain inhibition and possible collateral inhibition exerted between systems.

This dissertation is dedicated to my loving parents, Arthur and Reba Sperber who gave their children everything. And to my brother, Kenneth and my sister, Terri who were always there when I needed them.

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Research conducted over the past fifteen years has established the existence of multiple pain inhibitory systems in rodents. Some of these appear to be mediated by the endogenous opioids while others appear to act independently of the opioid system. A principal tool by which different pain inhibitory systems can be activated is to expose an animal to acute environmental stressors (see reviews: Bodnar, Kelly, Brutus & Glusman; Watkins & Mayer, 1982). Based upon the type of stressor employed, one can generate different profiles of analgesic reponses. Though considerable research has putatively involved the neurotransmitter acetylcholine (ACh) in opioid forms of pain inhibition, little is known of its interaction with the stress-related activation of pain inhibitory systems. Since the mucarinic receptor has been implicated primarily in cholinergic mediation of pain inhibition, the present study employed the potent muscarinic receptor antagonist, scopolamine, to determine if it altered the different analgesic responses of rats following acute exposure to cold-water swims (CWS), 2-deoxy-D-glucose (2DG) glucoprivation or two forms of inescapable footshock (IFS). Analysis of analgesic alterations across multiple pain tests and across the dose- and time-response curves of the tertiary (scopolamine) and quaternary (methylscopolamine) forms of the muscarinic receptor antagonist were performed. Alterations in the analgesic

stress responses were compared with the effects of scopolamine and methylscopolamine upon a) other stress-related responses, b) analgesia induced by the muscarinic receptor agonist (pilocarpine), c) basal pain thresholds and d) analgesia induced by the opiate mu receptor agonist, morphine, the opiate delta receptor agonist, D-ala-d-leu-enkephalin (DADL) and the opiate epsilon receptor agonist, beta-endorphin (BEND). The following sections review evidence for a) the existence of an opioid mediated pain inhibitory system, b) interactions between opiate and cholinergic analgesic systems, c) the existence of multiple pain inhibitory systems activated by environmental stressors and d) a rationale for the present series of experiments.

A. An Opioid Pain Inhibitory System

Following the formulation of the gate control theory of pain (Melzack & Wall, 1965), subsequent research has focused on that aspect of the theory which suggested the existence of centrifugal influences from the brain to the spinal cord specifically designed to inhibit pain. This was confirmed by initial observations that either morphine microinjections into or electrical stimulation of discrete subcortical areas induced analgesia (Reynolds, 1969; Tsou & Jang, 1964). Both morphine microinjection analgesia

(MMA) and stimulation-produced-analgesia (SPA) were found to be elicited from midline subcortical structures, including the midbrain dorsal raphe nucleus and the periaqueductal grey (PAG), the hypothalamic and pontine periventricular grey areas, the medullary nucleus raphe magnus (NRM), the nucleus reticularis gigantocellularis (NRGC) and the ventral thalamus (see reviews; Gebhart, 1982; Mayer & Price, 1976; Messing & Lytle, 1977; Yaksh & Rudy, 1978).

Several other similarities between MMA and SPA suggest they activate a common pain inhibitory system. First, summation of subanalgesic morphine doses and subanalgesic stimulation current produced analgesia (Kelly, Such, Brutus, Glusman & Bodnar, 1978; Samanin & Valzelli, 1971). Second, SPA and MMA result in tolerance with repeated stimulations (Jacquet & Lajtha, 1975; Mayer & Hayes, 1975; Sessle, Dubner, Greenwood & Lucien, 1975) and partial cross-tolerance developed with these two manipulations (Mayer & Hayes, 1975). Third, lesions placed in either the NRM or the dorsolateral funiculus (the descending projection to the dorsal horn of the spinal cord), disrupted both forms of analgesia (Basbaum, Morley, O'Keefe & Clanton, 1977; Dostrovsky & Deakin, 1977; Rhodes, 1979; Samamin, Ghezzi, Mauron & Valzelli, 1973). Fourth, both MMA and SPA are decreased following either depletion of serotonin or dopamine or receptor blockade and are in-

creased following norepinephrine depletion (Akil & Liebeskind, 1975; Akil & Mayer, 1972; Hughes et al., 1977; Tenen, 1968; Vogt, 1974; Yaksh & Rudy, 1978). Fifth, the analgesic responses following MMA and SPA elicited from the PAG produced excitation of units in the NRM and inhibition of nociceptive units in the substantia gelatinosa of the dorsal horn of the spinal cord (Behbehani & Pomeroy, 1978; Fields & Anderson, 1978; Oliveras, Besson, Guilbaud & Liebeskind, 1974). Further, this inhibition of nociceptive input by MMA and SPA was selective in that non-nociceptive units in the dorsal horn were not affected (Fields & Basbaum, 1978; LeBars, Menetrey, Conseiller & Besson, 1975; Liebeskind, Guilbaud, Besson & Oliveras, 1973; Lovick & Wolstencroft, 1979; Oliveras et al., 1974; Satoh & Takagi, 1971; Willis, Haber & Martin, 1977). Finally, endogenous opioid peptides and opiate receptors were found in high concentrations in areas which elicited MMA and SPA (Atweh & Kuhar, 1977a, Sar et al., 1978; Mayer & Price, 1976; Watson et al., 1978; Yaksh & Rudy, 1978).

The latter findings suggest a physiological substrate through which MMA and SPA exert their analgesic effects with morphine activating opiate receptors and electrical stimulation producing release of endogenous opioids. These data are further supported by the observation that three types of opioid peptides, the endorphins, the en-

kephalins and the dynorphins (see review: Akil et al., 1984) also elicit analgesia. Low doses of BEND administered intracerebroventricularly (ICV) induced analgesia (Bloom et al., 1976; Jacquet & Marks, 1976; Ronai, Szekely, Graf, Dunai-Kovacs & Bajusz, 1976; Segal, Browne, Bloom, Ling & Guilleman, 1977) and higher ICV doses of the enkephalins produced a mild transient analgesia (Beluzzi, Grant, Garsky, Sarantakis, Wise & Stein, 1976; Graf, Szekely, Ronai, Dunai-Kovacs & Bajusz, 1976). In contrast, anti-sera raised against either met-enkephalin or BEND produced hyperalgesia (Bodnar, Nilaver, Wallace, Badillo-Martinez & Zimmerman, 1984). Dynorphin-related peptides typically elicited analgesia only after intrathecal injections into the subarachnoid space of the spinal cord (Basbaum, Jacknow, Mulcahy & Levine, 1983; Goldstein, 1979; Han & Xie, 1982; Piery, Lahti, Schroeder, Einspar & Barsuhn, 1982). Repeated administration of either endorphins or enkephalins resulted in tolerance and cross-tolerance with each other (Huidobro-Toro & Way, 1980) or with morphine (Szekely, Ronai, Dunai-Kovacs, Miglecy, Bajusz & Graf, 1977; Van Ree, DeWied, Bradbury, Hulme, Smythe & Snell, 1976; Wei & Loh, 1976).

It should be noted that the analgesic effects elicited by opiates or opioid peptides are not entirely homogeneous. For instance, opiate receptors have been localized in the spinal cord (Atweh & Kuhar, 1977c) and appear

to mediate analgesia following intrathecal injections (see review: Yaksh, 1981). This opiate spinal system interacts with, but is not totally dependent upon, supraspinal opiate systems (Yeung & Rudy, 1980a,b). Furthermore, the different groups of opioid peptides have been hypothesized to interact with different sub-populations of opiate receptors: mu, delta, kappa, sigma and epsilon (Herz et al., 1980; Lord et al., 1977; Martin et al., 1976). Various opioid peptides and opiate receptor subtypes appear to be differentially distributed in spinal and supraspinal structures (see review: Akil et al., 1984). In this regad, interactions among opiate and opioid agents sometimes produce unexpected results. For example, while simultaneous administration of BEND and morphine result in a potentiation of analgesic affects, simultaneous administration of enkephalins and morphine produce an attenuation of analgesic responses Huidobro-Toro, 1980). These data indicate that different opioid subsystems mediating pain inhibition exist. Based on such data, Fields and Basbaum (1978) proposed the existence of an opioid pain inhibitory system which was dependent upon descending bulbo-spinal pathways which received input from the PAG and other midbrain and pontine nuclei (Basbaum & Fields, 1984). An integral component of this system is the medullary midline cells of the NRM and NRGC which are directly activated by spinal input or indirectly activated through opioid-medi-

ated excitation from the PAG. In turn, these medullary units descend through the DLF to the dorsal horn and inhibit nociceptive-specific cells. Basbaum and Fields (1984) implicated two neurotransmitters, serotonin and norepinephrine in the mediation of these spinal effects (see also Yaksh, 1981). However, additional research (see below) has suggested that supraspinal opiate analgesia can also interact with the cholinergic system.

B. Acetylcholine and Analgesic Processes

Cholinergic agonists produce analgesia in humans (Floodmark & Wrammer, 1945; Pellanda, 1933) and animals (Davis, Pollock & Stone, 1932) across a wide variety of pain tests (Chen, 1958; Hendershot & Forsaith, 1959; Herz, 1962; Jacob & Barthelemy, 1965; Leslie, 1969; Metys, Wagner, Metsova & Herz, 1969). Tail-flick latencies, a measure of spinally-mediated noxious thermal reactivity (D'Amour & Smith, 1941; Grossman et al., 1982), were elevated following administration of either cholinesterase inhibitors (e.g. physostigmine), muscarinic receptor agonists (e.g. pilocarpine), nicotinic receptor agonists (i.e. nicotine) or ACh itself (Battacharayer & Nayak, 1978; Bhargava & Way, 1972; Calcutt & Spence, 1972; Dayton & Garrett, 1973; Harris, Dewey & Howes, 1968; Harris, Dewey, Howes, Kennedy & Pars, 1969; Ireson, 1970; Paalzow

& Paalzow, 1975; Pedigo & Dewey, 1981; Romano & King, 1980; Sahley & Bernston, 1979). Furthermore, both cholinergic receptor agonists and ACh produced analgesia on the writhing test, a measure of reactivity to noxious visceral stimuli (Dewey, Chau-Phen, Cocolas, 1976; Ireson, 1970; Parkes & Pike, 1965; Pedigo, Dewey & Harris, 1975), the hot-plate test (Mattila, Ahtee, & Saarinvaara, 1968; Pleuvry & Tobias, 1971; Romano & King, 1981; Van Eick & Bock, 1971) and the jump test (King & Romano, 1980; Pert, 1975) in a manner similar to morphine. Muscarinic receptor antagonists, particularly scopolamine and atropine, blocked analgesia induced by cholinergic receptor agonists (Dayton & Garrett, 1973; Ireson, 1970; King & Romano, 1981; Pedigo et al., 1975; Sahley & Benston, 1979).

More recently, studies have focused upon the mechanism of action between acetylcholine and morphine induced analgesia. While morphine analgesia was potentiated by simultaneous adminstration of either ACh (Pedigo et al., 1975) or cholinomimetics (Bhargava & Way, 1972; Calcutt & Spence, 1972; Howes et al., 1969; Ireson, 1970; Kakkola & Ahtee, 1977; Pleuvry & Tobias, 1971; Takemori, Tulunay, & Yano, 1975), cholinergic analgesia was potentiated by prior administration of opiates (Romano & King, 1976). Like morphine, ACh and cholinomimetics developed tolerance following repeated administrations (Dayton & Garrett, 1973; Howes et al., 1969; Little & Rees, 1974; Pedigo &

Dewey, 1981; Pert, 1975).

If the cholinergic and opiate analgesic systems interact physiologically with one another, it would be expected that cholinergic and opiate receptor antagonists would block the other type of analgesia, in addition, cross-tolerance should develop between the analgesic effects. Data supporting this hypothesis have not been entirely consistent. Some studies reported that cholinergic receptor antagonists partially attenuated morphine analgesia (Romano & King, 1976; Takemori et al., 1975) and opiate receptor antagonists partially blocked cholinergic analgesia (Harris et al., 1968; Howes et al., 1969; Lewis, Cannon & Liebeskind, 1981; Pedigo & Dewey, 1981; Rambadran & Jacobs, 1978; Romano & King, 1981) while others have failed to observe opiate antagonism of or by cholinergic analgesia (Dayton & Garrett, 1973; Ireson, 1970; Kakkola & Ahtee, 1977; Pleuvry & Tobias, 1971). However, still others have found a potentiation of morphine analgesia following cholinergic antagonists (Lewis, Cannon & Liebeskind, 1981). Repeated administration of cholinergic receptor agonists produced only partial cross-tolerance with morphine analgesia (Pedigo & Dewey, 1981) while morphine-tolerant monkeys displayed normal cholinergic analgesia (Pert & Maxey, 1975). Finally, analgesic cross-tolerance between morphine and ACh fails to occur in rats (Little & Rees, 1974; Howes et al., 1969). Thus,

while cholinergic and opiate agents each produce analgesia and potentiate each other's analgesic effects, it is not clear whether the latter actions are the result of activation of common pain-inhibitory mechanisms.

C. Multiple Pain Inhibitory Systems

The heterogeneity of opioid forms of pain inhibition and the apparent heterogeneity of cholinergic and opiate forms of pain inhibition correspond with other evidence supporting the heterogeneity of pain inhibitory processes. For instance, decreases in analgesia induced by acupuncture, but not hypnosis occur following pretreatment with opiate receptor antagonists (see review: Mayer & Price, 1976). Naloxone, an opiate receptor antagonist, reduced SPA elicited from ventral but not dorsal PAG sites (Akil et al., 1976; Cannon et al., 1983; Oliveras et al., 1977; Pert et al., 1976; Prieto et al., 1983; Yaksh et al., 1976). Moreover, receptor antagonists of vasopressin and opiates failed to block each others analgesia (Berkowitz & Sherman, 1982; Berntson & Berson, 1980; Kordower & Bodnar, 1984). Furthermore, neurotensin analgesia is neither cross-tolerant with morphine nor reversed by naloxone (Clineschmidt et al., 1977; Osbahr et al., 1981).

The primary focus of the present study involves the heterogeneous pain inhibitory processes activated by acute

exposure to several environmental stressors or stress-induced analgesia, SIA, (see reviews: Amir et al., 1980; Bodnar et al., 1980c; Terman et al., 1984; Watkins & Mayer, 1982). Different stressors or different parameters of the same stressor can elicit analgesia that exhibit independent physiological or pharmacological profiles. Differences in stress-related analgesic responses are observed following a) opiate receptor antagonists or morphine tolerance, b) manipulations of the hypothalamo-hypophyseal-adrenal axis or c) physiological and pharmacological manipulations of central nervous system processes. In this regard, Watkins and Mayer (1982) classified stress-related analgesic responses as either: opioid-neural, opioid-neurohormonal, nonopioid-neural, or nonopioid- neurohormonal. A stress-related analgesic response representative of each category was chosen to assess cholinergic mediation and a review of the underlying mechanisms of each follows: cold-water swims (CWS: nonopioid-neurohormonal), 2-deoxy-D-glucose (2DG: opioid-neurohormonal), inescapable shock delivered to the forepaws (FPS: opioid-neural) and inescapable shock delivered to the hindpaws (HPS: neural-nonopioid).

1. Cold-Water Swim (CWS) Analgesia

Acute exposure to CWS (2-15°C for 3.5 min) produces

analgesia for up to 120 min as measured by several nociceptive tests (Bodnar, Glusman, Brutus, Spiaggia & Kelly, 1979; Bodnar, Kelly, Spiaggia & Glusman, 1978). On the other hand, rats exposed to swims at warmer temperatures (28-35 C) fail to display analgesia (Bodnar et al., 1978c, Bodnar & Sikorszky, 1983). Repeated exposure to CWS over 14 days results in adaptation of the analgesic, but not the hypothermic responses (Bodnar et al., 1978d) suggesting that the analgesic response is specific to the swim's stressful consequences and not merely a function of either water immersion, hypothermia, dampness, forced exercise or fatigue.

CWS analgesia has been categorized as nonopioid-neurohormonal for the following reasons. First, CWS analgesia and morphine analgesia failed to develop cross tolerance (Bodnar, Kelly, Steiner & Glusman, 1978). Second, naloxone (1-20 mg/kg) although capable of eliminating morphine analgesia (Yaksh & Rudy, 1978), failed to significantly alter CWS analgesia (Bodnar, Kelly, Spiaggia, Ehrenberg & Glusman, 1978). Third, the putative anti-enkephalinase, D-phenylalanine, increased morphine analgesia, and decreased CWS analgesia (Bodnar, Lattner & Wallace, 1980). Pretreatment with the irreversible high-affinity opiate receptor antagonist, naloxazone (Pasternak et al., 1980), decreased morphine analgesia and increased CWS analgesia (Kirchgessner, Bodnar & Pasternak, 1982). These data

indicate that CWS analgesia appears to act independently of opioid mechanisms for the expression of its analgesic response and may in the course of its activation, inhibit opioid processes through a proposed process of collateral inhibition (see review: Bodnar, 1984).

A neurohormonal component of CWS analgesia is also evident in that hypophysectomy decreased CWS analgesia (Bodnar et al., 1979) and adrenalectomy potentiated CWS analgesia (Glusman, Bodnar, Mansour & Kelly, 1980). A role for the adrenocortical system in CWS analgesia has been postulated since posterior lobectomy failed to alter CWS analgesia (Glusman, Bodnar, Kelly, Serio, Stern & Zimmerman, 1979) thereby suggesting that the anterior lobe of the pituitary gland was responsible for the effect. Second, adrenal demedullation and peripheral catecholamine depletion failed to alter CWS analgesia (Bodnar, Sharpless, Kordower, Potegal & Barr, 1982) suggesting that the adrenal cortex was responsible for the effect. Third, stimulation of glucocorticoids with dexamethasone decreased CWS analgesia (Marek et al., 1982) and acted to inhibit subsequent release of adrenocorticotrophic hormone from the anterior lobe of the pituitary gland (Krieger & Liotta, 1979). Finally, corticosteroid synthesis inhibition with metyrapone increased CWS analgesia (Mousa et al., 1981). Whether the hormonal component of CWS analgesia initially triggers and/or subsequently maintains the

response is not yet known. However, it appears that the hormonal component of CWS analgesia is dependent upon the integrity of the hypothalamo-hypophyseal axis (Krieger & Liotta, 1979) for its expression since destruction of the medial basal hypothalamus following neonatal administration of monosodium glutamate (MSG :Olney, 1969) decreased CWS analgesia (Badillo-Martinez et al., 1984b; Bodnar et al., 1980a). Again, CWS analgesia and morphine analgesia dissociate since hypophysectomy increased morphine analgesia (Bodnar et al., 1979a; Holaday, Law, Loh & Li, 1978).

Evidence for a neural component in the mediation of CWS analgesia is derived largely from neuropharmacological manipulations. A role for norepinephrine in CWS analgesia is supported by its potentiation following pretreatment with either the noradrenergic receptor stimulant, clonidine (Bodnar, Merrigan & Sperber, 1983) or the noradrenergic reuptake inhibitor, desipramine (Bodnar, Mann, & Stone, 1985), and its reduction following lesions placed in the noradrenergic locus coeruleus (Bodnar et al., 1980d). Reciprocal effects upon systemic morphine analgesia occur following noradrenergic manipulations (see review: Yaksh & Rudy, 1978). A role for dopamine in CWS analgesia is supported by its reduction following the dopamine receptor agonist, apomorphine (Bodnar et al.,

1980b) and its potentiation following neuroleptics (Bodnar & Nicotera, 1982).

While decreases in serotonin levels attenuate morphine analgesia (see review: Messing & Lytle, 1977), inhibition of serotoinin synthesis with parachlorophenylalanine (PCPA: Bodnar, Kordower, Wallace & Tamir, 1981) or lesions placed in the serotinergic dorsal raphe nucleus (Brutus, Kelly, Glusman & Bodnar, 1979) failed to alter CWS analgesia. A role for vasopressin in CWS analgesia is supported by its reduction in vasopressin-deficient (Valtin, 1962) Brattleboro rats (Bodnar, Zimmerman, Nilaver, Mansour, Thomas, Kelly & Glusman, 1980) and in rats with lesions placed in the hypothalamic paraventricular nucleus (Bodnar, Truesdell & Nilaver, 1986).

2. 2-Deoxy-D-Glucose (2DG) Analgesia

Acute administration of 2DG induces analgesia (Bodnar, Kelly, Brutus, Mansour & Glusman, 1978), in addition to hyperphagia, glucoprivation, hyperglycemia, peripheral sympatho-medullary and pituitary-adrenal discharge (Brown, 1962; Himsworth, 1970; Wick, Drury, Nakada & Wolfe, 1957). 2DG analgesia displayed adaptation with repeated administration and exhibited both cross-tolerance and synergy with morphine analgesia (Bodnar, Kelly & Glusman, 1979; Spiaggia et al., 1979). Yet 2DG analgesia was not affec-

ted by naloxone pretreatment (1-20 mg/kg) suggesting that the opiate and 2DG interaction does not occur at the level of the opiate receptor (Bodnar et al., 1979b). Like morphine analgesia, 2DG analgesia was potentiated by hypophysectomy (Bodnar, Kelly, Mansour & Glusman, 1979) and was also increased in MSG-treated rats (Badillo-Martinez et al., 1984), indicating that 2DG and CWS analgesia are differentially affected by hormonal manipulations. However, 2DG and CWS analgesia displayed full and reciprocal cross-tolerance and both were potentiated by dopamine receptor antagonists (Bodnar & Nicotera, 1982), decreased by dopamine receptor stimulation (Bodnar, Kelly, Brutus, Greenman & Glusman, 1980) and were unaffected by PCPA (Bodnar et al., 1981a). Like morphine analgesia, lesions placed in the PAG reduced 2DG analgesia (Brutus et al., 1979; see review: Yaksh & Rudy, 1978).

3. Inescapable Footshock (IFS) Induced Analgesia

Acute exposure to IFS elicits an analgesic response which lasts from a few minutes to several hours depending upon the parameters employed (Madden, Akil, Patrick & Barchas, 1977). In contrast, acute exposure to equivalent amounts of escapable footshock elicited little or no analgesia (Jackson, Maier & Coon, 1979; Maier, Drugan & Grau, 1982). Like CWS and 2DG analgesia, repeated expo-

sure to IFS results in adaptation of the analgesic responses (Madden et al., 1977; Mah, Suissa & Anisman, 1980). Three different IFS procedures have demonstrated that opioid-mediated and nonopioid-mediated analgesia can be elicited depending upon the specific parameters of IFS. First, naloxone pretreatment or morphine tolerance reduced prolonged intermittent footshock analgesia, but failed to affect brief continuous footshock analgesia (Lewis, Cannon, & Liebeskind, 1980; Lewis, Sherman & Liebskind, 1981). Second, analgesia induced by 60 or 80 but not by 20 tailshocks, was reduced by opiate receptor antagonists (Grau et al., 1981). Third, shock delivered to the forepaws (forepaw shock: FPS) but not to the hindpaws (hindpaw: HPS) elicited analgesia that was dependent upon opioid processes (Watkins & Mayer, 1982; Watkins, Cobelli, Faris, Aceto & Mayer, 1982). However, since the first two procedures appear to be dependent upon neurohormonal processes for their analgesic effect (Lewis et al., 1981, 1982; MacLennan, Drugan, Hyson, Maier, Madden & Barchas, 1982), this section and the subsequent experiments will focus upon the neural substrate mediating FPS and HPS analgesia.

FPS, but not HPS analgesia, appears to be opioid-mediated since it is cross-tolerant with morphine (Watkins & Mayer, 1982) and is decreased by either peripheral or central naloxone pretreatment (Cobelli, Watkins & Mayer,

1980; Watkins & Mayer, 1982). Neither FPS nor HPS analgesia appear to possess hormonal components since hypophysectomy or dexamethasone pretreatment fail to alter either response (Watkins, Cobelli, Newsome & Mayer, 1982b). Furthermore, adrenal and sympatho-medullary function do not appear to be involved in FPS or HPS analgesia since neither form of analgesia is affected by adrenalectomy or inhibition of sympathetic noradrenergic release (Watkins et al., 1982b).

Both FPS and HPS analgesia have neural components which differ from each other. Although dorsal lateral funiculus (DLF) lesions and spinal transections disrupt both forms of footshock analgesia, HPS analgesia appears to act through an intraspinal component (Watkin et al., 1984b) while intrathecal naloxone pretreatment blocks FPS analgesia (Watkins & Mayer, 1982). The medullary raphe nuclei appear to be the origin of the spinal influences mediating FPS analgesia; HPS analgesia is only partially mediated by this pathway (Watkins, Johannessan, Kinscheck & Mayer, 1984c). Neither form of footshock analgesia is affected by PAG lesions or decerebration suggesting that midbrain and forebrain structures are not involved in mediating forepaw or hindpaw footshock analgesia (Watkins, Kinscheck & Mayer, 1984b).

Rationale

The present experiments examined the role of the muscarinic receptor in SIA through the use of scopolamine and methylscopolamine. Previous research has established a role for ACh in different types of SIA. Romano and King (1980) found that hemicholinium and cholinergic receptor antagonists, atropine, hemicholinium and bencytazine attenuated CWS analgesia (a nonopoiod analgesia) on the tail-flick and jump tests, while only bencytazine blocked CWS analgesia on the hot-plate test. Scopolamine reduced analgesia induced by opioid-mediated prolonged intermittent footshock, but not analgesia induced by non-opioid-mediated brief continuous footshock (Lewis et al., 1980; 1981). Finally, scopolamine also blocked the opioid-sensitive effects of another form of prolonged IFS analgesia (MacLennan et al., 1983).

The first experiment examined the effects of scopolamine and methylscopolamine upon analgesia elicited by four stressors with different pharmacological and physiological profiles: CWS (nonopioid-neurohormonal), 2DG (opioid-neurohormonal), FPS (opioid-neural) and HPS (nonopoid-neural). In the assessment of CWS and 2DG analgesia, two pain tests were employed: the spinally-mediated tail-flick test (D'Amour & Smith, 1941; Grossman et al.,

1982) and the supraspinally-mediated jump test (Evans, 1961). Since IFS produces hyperalgesia on the jump test as a result of sensitization (Kelly, 1982), only the tail-flick test was employed to assess IFS analgesia.

The first experiment also examined the effects of scopolamine and methylscopolamine upon other stress responses following CWS and 2DG since muscarinic receptor manipulations may also alter other physiological stress responses (e.g., hypothermia and hyperphagia) through its parasympathetic actions (Carlson, 1981). These controls examined the possibility that any muscarinic-mediated changes in SIA could be due to epiphenomenological alterations in other stress-related responses and not the direct result of modifications in endogenous pain inhibitory systems.

CWS hypothermia was examined because it displays both similarities and differences with CWS analgesia. CWS analgesia, but not CWS hypothermia, was reduced following repeated exposure to CWS (Bodnar et al., 1978d), in hypophysectomized animals (Bodnar et al., 1979c) and in animals pretreated with D-phenylalanine (Bodnar et al., 1980d) and was increased following desipramine (Bodnar et al., 1985). In contrast, both CWS analgesia and CWS hypothermia were reduced in MSG-treated rats (Badillo-Martinez et al., 1984) and increased in clonidine-treated rats (Bodnar et al., 1983b).

Dissociations between 2DG analgesia and 2DG hyperphagia have also been observed. 2DG analgesia, but not 2DG hyperphagia was decreased following repeated pre-treatment with either 2DG or morphine (Bodnar et al., 1978b; Bodnar et al., 1979b; Bodnar, Kramer, Simone, Kirchgessner & Scalisi, 1983). 2DG hyperphagia, but not analgesia was decreased following either naloxone pre-treatment (Bodnar et al., 1979b), lesions placed in the lateral hypothalamus or zona incerta (Bodnar et al., 1983a), hypophysectomy (Bodnar et al., 1979a) or neonatal MSG treatment (Badillo-Martinez et al., 1982).

Tertiary cholinergic compounds (e.g. scopolamine) are able to cross the blood-brain-barrier and therefore have both central and peripheral effects in contrast to quar-ternary cholinergic compounds (e.g. methylscopolamine) which typically do not have access to the brain and have only peripheral effects. By comparing the effects of tertiary and quarternary cholinergic agents, it is possible to determine the relative potency of centrally and peri- pherally acting drugs. Scopolamine was selected because a comparison of sixteen cholinergic antagonists (Parkes, 1965) revealed that the peripheral activity of scopolamine was similar to other muscarinic agents while its central activity was ten times greater than other muscarinic agents.

Since it was important to have a standard with which

to compare scopolamine's effect on SIA, the second experiment examined the analgesic properties of the muscarinic receptor agonist, pilocarpine and the ability of scopolamine and methylscopolamine to block pilocarpine-induced analgesia. Furthermore, manipulation of the muscarinic receptor may conceivably produce changes in analgesic responses to stress by merely shifting baseline pain thresholds. Moreover, a similar shift in baseline food intake or core temperature by either scopolamine or methscopolamine could influence 2DG hyperphagia and CWS hypothermia. Therefore, the effects of scopolamine and methylscopolamine upon baseline measures of tail-flick latencies, jump thresholds, food intake and core body temperatures were examined in the third experiment.

The heterogeneity of the opioid receptor subtypes into mu1, mu2, kappa, delta, epsilon and sigma has been characterized by their selective sensitivity to different opiate drugs and behavioral responses (Lord et al., 1977; Martin et al., 1976; Pasternak, 1980). While the mu1 receptor is believed to be the primary mediator of supraspinal analgesia (Pasternak et al., 1980), DADL and BEND appear selective for delta and epsilon receptors in the spinal cord (Atweh & Kuhar, 1977a,b,c; Schmauss & Yaksh, 1984). Since the interaction of the different opiate receptors with the cholinergic system has not been characterized and since different endogenous opioids have

different behavioral characteristics from morphine (see review: Akil et al., 1984), the fourth experiment examined scopolamine's effects upon analgesia induced by various opioid drugs including morphine, DADL and BEND analgesia.

General Method

Subjects: Adult male Sprague Dawley rats (3-12 mo) were bred in the animal colony at Queens College. The colony was originally derived from rats obtained from the Charles River Breeding Laboratories. Animals were housed two to a cage (23 cm x 26 cm x 20 cm) and maintained on a 12h light: 12h dark cycle with food (Purina Rat Chow) and water available ad libitum.

Drugs: Scopolamine hydrobromide (Sigma), scopolamine methyl nitrate (Sigma), 2-deoxy-D-glucose (Sigma), pilocarpine hydrochloride (Sigma), morphine sulfate (Pennick) and naloxone hydrochloride (Endo Laboratories) were all dissolved in normal physiological saline in injection volumes of 1 ml/kg body weight. The drugs were all administered intraperitoneally (IP) with the exception of morphine which was administered subcutaneously (SC). D-ala-D-leu enkephalin and beta-endorphin (Peninsula Laboratories) were dissolved in normal physiological saline and were injected intracerebroventricularly (ICV) in a 5 ul volume at the rate of 1 ul/15 sec. A 28 gauge internal cannula, connected to a microsyringe (Hamilton) by PE-50 tubing (Clay Adams) was used to infuse the DADL and BEND into the lateral ventricle.

Surgery: Those rats receiving ICV injections (Experiment 4) were anesthesized with chlorpromazine hydrochloride (3 mg/ml normal saline/kg body weight, IP) followed 15 min later by ketamine hydrochloride (95 mg/ml sterile water/kg body weight, IM). A 22 gauge guide cannula (Plastic Products) was stereotaxically (Kopf) implanted into the left lateral ventricle. The incisor bar was set at +5 mm and the stereotaxic coordinates were 0.5 mm anterior to the bregma suture, 1.3 mm lateral to the mid-sagittal suture and 3.6 mm from the top of the skull. The cannula was anchored to three stainless steel jewelers screws with dental acrylic. Behavioral testing began ten days after recovery from surgery.

Histology: Upon completion of the behavioral testing those animals receiving cannulae (Experiment 4) were anesthesized with sodium pentobarbitol (100 mg/2 ml, IP) and decapitated. The brains were extracted and fixed in 10% formalin for one week. The brains were then coronally sectioned through the lateral ventricle and visually inspected for accurate cannula placement. Only those animals with correct placements were included in the data analysis.

Core Body Temperature: Core body temperature was determined by inserting a rectal probe of a digital ther-

mometer (Bailey Instrument) until a stable reading (within Ø.1 C) was achieved.

Tail Flick Test: Tail flick latencies were measured by a radiant heat source (IITC Company). The heat source was applied 2-5 cm from the tip, of the dorsal surface, of the rat's tail. The onset of the heat stimulus initiated an electronic timer (in sec) which was terminated when the rat moved its tail and exposed a photocell. Latencies of the response were accurate to the nearest Ø.Ø1 sec. If the animal failed to respond, the trial was terminated after 6 sec (Experiments 1-3) or 1Ø sec (Experiment 4) to avoid tissue damage. Blocks of three trials were administered with intertrial intervals of 1Ø sec.

Jump Test: Electric shocks were delivered by a 6Ø Hz constant current shock generator (BRS/LVE) through a grid scrambler (Campden Instruments) to a 3Ø cm X 24 cm floor composed of 16 grids. Shock thresholds were determined according to an ascending method of limits procedure (Evans, 1961). The ascending component of the method of limits was employed to prevent the animal from receiving supra-threshold shock since such exposure can alter thresholds on subsequent pain tests (e.g. tail-flick test: Kelly, 1982). The jump threshold was defined in mA as the lowest of two consecutive intensities that elicited

simultaneous withdrawal of the hindpaws from the grid floor. Each of the six trials was initiated with a 300 msec duration footshock delivered at a current intensity of 0.1 mA. Subsequent shocks were delivered at 10 sec intervals and were increased in increments of 0.05 mA steps until the jump threshold was obtained. Following each trial, the current intensity was reset to 0.1 mA until six trials were completed. The purpose of a static starting point in the jump threshold determinations was to insure consistency with other experiments. It also mimics the activation of heat delivered to the tail or feet with the tail-flick and hot-plate tests, respectively. Previous work in our laboratory has repeatedly shown that the use of a static starting point does not result in learning due to errors of anticipation. The tail-flick test preceeded the jump test to minimize carry-over effects from one pain test to another (Kelly, 1982). Group assignment was determined by matching animals on the basis of their baseline jump thresholds.

Food Intake: Animals were adapted to individual cages with food and water ad libitum 12-16 hrs prior to ingestion testing. Testing began at 1 to 4 hrs into the light cycle when fresh food pellets (30-40 g: Purina Rat Chow) were placed in each cage. After 6 hrs, total food consumption was measured by calculating the amount of

remaining food and adjusting for spillage. Group assignment was determined by matching animals on the basis of their body weight.

Footshock: Electrical shock was continuously delivered at 1.6 mA for 90 sec (see Watkins & Mayer, 1982). In the FPS condition, a shoelace was tied around the rats lower torso, anterior to its hip so that the caudal end of the body was raised from the grids. In the HPS condition, a shoelace was tied around the upper torso above the chest so that the rostral part of the body was raised during shock exposure.

Statistical Analysis: A split-plot ANOVA (Kirk, 1968) was employed to analyze the main effect of the different drugs over time on each dependent measure (jump thresholds, tail-flick latencies, body temperature and food intake) and the interaction between and among these variables. In all experiments Dunnett test (Keppel, 1973) were performed on the experimental scores at each test time with the two control groups. The first series of comparisons employed the vehicle no-manipulation condition in order to determine whether an analgesic effect was present or not. The second series of Dunnett tests compared the vehicle-manipulation condition with all experimental drug treatments to determine whether the

analgesic condition was affected by scopolamine and methylscopolamine.

Experiment 1A: Muscarinic Receptor Antagonists and CWS Analgesia

Method

Ninety-four rats, matched for baseline jump thresholds were divided into the following groups: vehicle (n=8), vehicle (n=6)*, scopolamine at doses of 0.001 (n=6)*, 0.01 (n=8), 0.05 (n=6)*, 0.1 (n=8), 0.5 (n=6)*, 1.0 (n=8), and 10 mg/kg (n=8), methylscopolamine at doses of 0.01 (n=6)*, 0.1 (n=6)*, 1.0 (n=8) and 10.0 mg/kg (n=8). A 3.5 min swim at a water temperature of 2°C followed each injection by 5 min. An additional group (n=8) received a vehicle injection and no swim to serve as a no-stress control group. Core body temperatures, tail-flick latencies and jump thresholds were assessed prior to the injection and 30, 60, and 120 min after either the CWS or the no swim condition.

Results

CWS Analgesia (Jump Thresholds): Two separate analyses were performed (see * above) on the dose-response

* Examination of the data suggested that it was necessary to test additional drug doseages to provide a fuller dose-response curve. Therefore, these additional groups (*) were tested and analyzed separately.

functions involving: 1) the Ø.Ø1, Ø.1, 1.Ø and 1Ø mg/kg doses of scopolamine and the 1.Ø and 1Ø.Ø mg/kg of methlscopolamine and 2) the Ø.ØØ1, Ø.Ø5 and Ø.5 mg/kg doses of scopolamine and the Ø.Ø1 and Ø.1 mg/kg doses of methylscopolamine. In the first analysis, significant differences in jump thresholds were observed across test times ($F(3,168)=8.24$, $p<.ØØØ1$) and for the interaction between drug conditions and test times ($F(21,168)=1.88$, $p<.Ø16$). An analysis of the second group of animals revealed significant differences among groups ($F(6,37)=3.Ø9$, $p<.Ø15$) and across test times ($F(3,111)=5.88$, $p<.ØØØ9$). Table 1 presents the dose-response effects of the original doses of scopolamine and methylscopolamine upon CWS analgesia on the jump test. Table 2 displays the dose-response effects of the additional groups of scopolamine and methylscopolamine (see * on p. 27). For illustrative purposes, Table 1 (in appendix) shows the combined dose-response effects of all the doses of scopolamine and methylscopolamine. The results demonstrate two major overall effects: a) an elimination of CWS analgesia by scopolamine and methylscopolamine 6Ø min after the swim and b) a less consistent dose-dependent reduction in CWS analgesia by scopolamine and methyl-scopolamine 3Ø min after the swim.

Vehicle-treated rats displayed CWS analgesia on the jump test for up to 12Ø min following the swim. Rats

Table 1
Scopolamine and methylscopolamine effects upon cold-water swim (CWS) analgesia as measured by the jump test (mA).

DOSE (mg/kg)	CONDITION		POST-SWIM (min)			
			BL	30	60	120
Control						
0	No Swim	Mean	.306	.281	.305	.312
		SEM	.012	.015	.016	.028
0	CWS	Mean	.291	.463+	.399+	.390+
		SEM	.037	.040	.045	.040
Scopolamine						
0.01	CWS	Mean	.304	.353*	.365	.313
		SEM	.023	.066	.047	.043
0.1	CWS	Mean	.297	.274+*	.301*	.308
		SEM	.016	.024	.029	.013
1.0	CWS	Mean	.309	.382	.325	.308
		SEM	.014	.037	.033	.024
10.0	CWS	Mean	.306	.384+	.306*	.335
		SEM	.029	.044	.033	.020
Methylscopolamine						
1.0	CWS	Mean	.304	.328*	.333	.346
		SEM	.026	.043	.047	.035
10.0	CWS	Mean	.307	.423+	.345	.331
		SEM	.014	.066	.025	.023

Significantly higher (Dunnett comparison, $p<.05$) than 0/No Swim Condition (+) or lower than the 0/CWS Condition (*).

BL=baseline
SEM=standard error

treated with all but the lowest dose of scopolamine (0.001 mg/kg) failed to display CWS analgesia 60 min after the swim. Rats treated with all but the 0.1 mg/kg dose of methylscopolamine failed to display CWS analgesia 60 min after the swim. The 0.1 and 10.0 mg/kg doses of scopolamine significantly increased the magnitude of CWS analgesia at 60 min after the swim. Figure 1 illustrates the degree of CWS analgesia as a percent increase above the jump threshold values of vehicle-pretreated and unstressed rats. The right panel of Figure 1 illustrates the elimination of CWS analgesia by representative doses of scopolamine and methylscopolamine at 60 min after the swim. These data suggest that scopolamine and methylscopolamine pretreatment are reducing the duration of CWS analgesia on the jump test.

In assessing CWS analgesia on the jump test 30 min following the swim relative to vehicle-treated rats, significant reductions were observed following the 0.001, 0.01, 0.05, 0.1 and 0.5 mg/kg doses of scopolamine. In contrast, the 1.0 and 10.0 mg/kg doses of scopolamine failed to affect CWS analgesia on the jump test at this interval. Significant reductions in CWS analgesia were observed at 30 min following the swim in rats pretreated with the 0.01 and 1.0 mg/kg doses of methylscopolamine. Yet the 0.1 and 10.0 mg/kg doses of methylscopolamine failed to affect the analgesic response at this interval.

Table 2
The effect of additional doses of scopolamine and methyscopolamine pretreatment upon cold-water swim (CWS) analgesia as measured by the jump test (mA).

DOSE (mg/kg)	CONDITION		POST-SWIM (min)			
			BL	30	60	120
Control						
0	CWS (n=6)	Mean	.335	.421+	.379+	.415+
		SEM	.029	.048	.035	.023
Scopolamine						
0.001	CWS (n=6)	Mean	.334	.351	.486+	.432+
		SEM	.024	.039	.079	.051
0.05	CWS (n=6)	Mean	.343	.365	.321	.378
		SEM	.017	.024	.025	.021
0.5	CWS (n=6)	Mean	.304	.311	.302	.311
		SEM	.029	.022	.027	.039
Methylscopolamine						
0.01	CWS (n=6)	Mean	.268	.347	.304	.372
		SEM	.085	.040	.037	.038
0.1	CWS (n=6)	Mean	.317	.438+	.374	.434+
		SEM	.034	.063	.057	.048

Significantly higher (Dunnett comparison, $p<.05$) than 0/No Swim Condition (+) or lower than the 0/CWS Condition (*).

BL=baseline
SEM=standard error

The magnitude of the CWS effect was significantly greater for the 0.1 mg/kg dose of scopolamine at 30 and 60 min and for the 1.0 mg/kg dose of methylscopolamine at 30 min. The left panel of Figure 1 illustrates this effect for some representative doses of scopolamine and methylscoolamine. These data indicate that scopolamine and methylscopolamine reduce the duration and not the magnitude of CWS analgesia on the jump test.

CWS Analgesia (Tail-Flick Latencies): Again, two separate analyses were performed (see * p.27) on the dose-response function involving: 1) the 0.01, 0.1, 1.0 and 10 mg/kg doses of scopolamine and the 1.0 and 10.0 mg/kg doses of methylscopolamine and 2) the 0.001, 0.05 and 0.5 mg/kg doses of scopolamine and the 0.01 and 0.1 mg/kg doses of methylscopolamine. In the first analysis, significant differences in tail-flick latencies were observed among groups ($F(7,56)=11.41$, $p<.0001$), across test times ($F(3,168)=186.41$, $p<.0001$) and for the interaction between groups and times ($F(21,168)=4.51$, $p<.0001$). In the second analysis, significant differences were observed among groups ($F(6,37)=36.41$, $p<.0001$), across test times ($F(3,111)=130.96$, $p<0001$) and for the interaction between groups and test times ($F(3,111)=4.53$, $p<.0001$). Table 3 presents the dose-response effects of the original doses of scopolamine and methylscopolamine

Figure 1. Percent increase in jump thresholds (mA) at 30 and 60 min following cold-water swims (2°C for 3.5 min). Groups of eight rats each received either vehicle (0), scopolamine (S) at doses of 0.01, 0.1, 1.0 or 10.0 mg/kg (IP) or methylscopolamine (M) at doses of 1.0 or 10.0 mg/kg (IP) 5 min before the swim. The values were calculated as the percentage above the jump threshold of the vehicle-treated and unstressed rats (see Table 1). While S and M attenuated CWS analgesia in an irregular manner at 30 min after the swims, all doses eliminated CWS analgesia at 60 min following the swim. Significant differences from the 0/CWS condition (*) or the 0/no swim condition (✦).

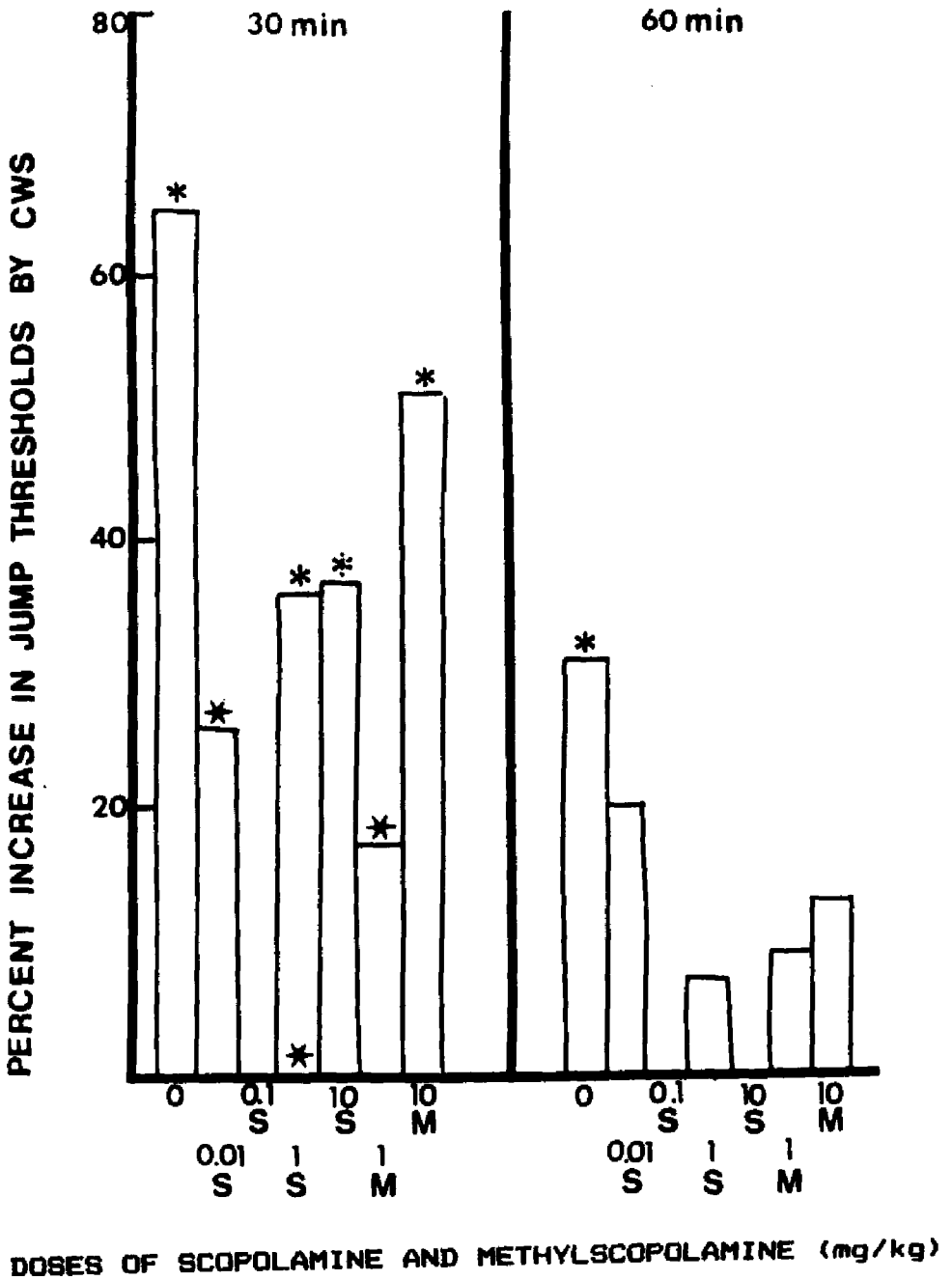
80
60
40
20
30 min
60 min
PERCENT INCREASE IN JUMP THRESHOLDS BY CWS
0
0.01 S
0.1 S
1 S
10 S
1 M
10 M
DOSES OF SCOPOLAMINE AND METHYLSCOPOLAMINE (mg/kg)

Table 3
Scopolamine and methylscopolamine effects upon cold-water swim (CWS) analgesia as measured by the tail-flick test (sec).

DOSE (mg/kg)	CONDITION		POST-SWIM (min)			
			BL	30	60	120
Control						
0	No Swim	Mean	2.30	2.39	2.42	2.34
		SEM	.121	.354	.116	.174
0	CWS	Mean	2.37	4.63+	3.69+	2.95
		SEM	.228	.213	.209	.182
Scopolamine						
0.01	CWS	Mean	2.36	5.10+	4.36+	3.56+
		SEM	.088	.191	.259	.177
0.1	CWS	Mean	2.77	5.10+	3.66+	3.14+
		SEM	.114	.232	.252	.223
1.0	CWS	Mean	2.39	4.83+	3.86+	3.33+
		SEM	.146	.209	.193	.124
10.0	CWS	Mean	2.32	4.73+	3.48+	3.11+
		SEM	.104	.204	.135	.119
Methylscopolamine						
1.0	CWS	Mean	2.68	4.93+	4.12+	3.90+*
		SEM	.161	.309	.402	.388
10.0	CWS	Mean	2.32	5.25+	4.43+*	4.01+*
		SEM	.212	.273	.309	.384

Significantly higher (Dunnett comparison, $p<.05$) than 0/No Swim Condition (+) or lower than the 0/CWS Condition (*).

BL=baseline
SEM=standard error

(see * p. 27) upon CWS analgesia on the tail-flick test. Table 4 displays the dose-response effects of the additional doses of scopolamine and methylscopolamine (see * p.27). For illustrative purposes, Table 2 (in appendix) shows the combined dose-response effects of all doses of scopolamine and methylscopolamine. Vehicle-treated rats displayed CWS analgesia on the tail-flick test for up to 120 min following the swim. Rats pretreated with all doses of scopolamine (0.001 to 10.0 mg/kg) and methylscopolamine (0.01 to 10.0 mg/kg) also displayed an analgesic response for up to 120 min after the swim. Methylscopolamine pretreatment significantly increased the magnitude of CWS analgesia on the tail-flick test at 60 min (10 mg/kg) and 120 min (1 and 10 mg/kg) following the swim relative to its time-matched vehicle control. Thus, test specific effects are observed for muscarinic receptor modulation of CWS analgesia: scopolamine and methylscopolamine reduce CWS anlgesia on the jump test but not on the tail-flick test.

CWS Hypothermia (Core Body Temperature): Two separate analyses were performed (see * p.27) on the dose-response functions involving: 1) the 0.01, 0.1, 1.0 and 10.0 mg/kg doses of scopolamine and the 1.0 and 10.0 mg/kg doses of methylscopolamine and 2) the 0.001, 0.05 and 0.5 mg/kg doses of scopolamine and the 0.01 and 0.1

Table 4
The effect of additional doses of scopolamine and methylscopolamine pretreatment upon cold-water swim (CWS) analgesia as measured by the tail-flick test (sec).

DOSE (mg/kg)	CONDITION		POST-SWIM (min) BL	30	60	120
Control						
Ø	CWS (n=6)	Mean	2.98	5.65+	4.60+	3.93+
		SEM	.123	.191	.236	.212
Scopolamine						
Ø.ØØ1	CWS (n=6)	Mean	3.62	5.81+	4.75+	4.25+
		SEM	.340	.101	.258	.108
Ø.Ø5	CWS (n=6)	Mean	3.23	5.88+	4.51+	4.51+
		SEM	.322	.182	.411	.198
Ø.5	CWS (n=6)	Mean	3.31	5.80+	4.85+	4.52+
		SEM	.160	.130	.265	.529
Methylscopolamine						
Ø.Ø1	CWS (n=6)	Mean	3.05	5.75+	4.47+	4.01+
		SEM	.231	.139	.366	.337
Ø.1	CWS (n=6)	Mean	2.38	5.72+	4.60+	3.93+
		SEM	.248	.163	.169	.246

Significantly higher (Dunnett comparison, $p<.05$) than Ø/No Swim Condition (+) or lower than the Ø/CWS Condition (*).

BL=baseline
SEM=standard error

mg/kg doses of methylscopolamine. In the first analysis, significant differences in core body temperature were observed among groups ($F(7,56)=4.84$, $p<.0001$), across test times ($F(3,168)=236.77$, $p<.0001$) and for the interaction between groups and test times ($F(21,168)=5.44$, $p<.0001$). In the second analysis, significant differences were observed among groups ($F(6,37)=11.73$, $p<.0001$), across test times ($F(3,116)=235.29$, $p<.0001$) and for the interaction between groups and test times ($F(18,111)=7.78$, $p<.0001$). Table 5 presents the dose-response effects of the original doses of scopolamine and methylscopolamine (see * p.27) upon CWS hypothermia. Table 6 displays the dose-response effects of the additional doses of scopolamine and methylscopolamine (see * p.27) upon CWS hypothermia. For illustrative purposes, Table 3 (in appendix) presents the combined effects of all doses of scopolamine and methylscopolamine upon CWS hypothermia. Vehicle-treated rats displayed CWS hypothermia for up to 30 and 60 min following the swim. Rats treated with several low doses of scopolamine (0.001, 0.01, 0.05 and 0.5 mg/kg) and methylscopolamine (0.01 mg/kg) demonstrated CWS hypothermia which lasted for up to 120 min. Indeed, significant increases in the magnitude of CWS hypothermia were observed following scopolamine at doses of 0.01 mg/kg (30-120 min) and 1 mg/kg (30-60 min) and methylscopolamine at a dose of 10 mg/kg (60 min). These data indicate that

Table 5
Scopolamine and methylscopolamine effects upon cold-water swim (CWS) hypothermia (°C).

DOSE (mg/kg)	CONDITION		POST-SWIM (min)			
			BL	30	60	120
Control						
0	No Swim	Mean	36.7	36.3	36.7	36.7
		SEM	.285	.604	.284	.349
0	CWS	Mean	37.3	29.9+	34.6	35.9
		SEM	.133	.767	.564	.409
Scopolamine						
0.01	CWS	Mean	37.1	27.3+*	31.2+*	33.8+*
		SEM	.228	1.38	1.37	1.11
0.1	CWS	Mean	36.8	29.0+*	34.1+	35.6
		SEM	.239	.854	1.00	.656
1.0	CWS	Mean	36.9	27.6+*	32.6+*	35.3
		SEM	.334	.575	.918	.515
10.0	CWS	Mean	36.7	30.1+	33.4+	35.9
		SEM	.424	1.35	.930	.374
Methylscopolamine						
1.0	CWS	Mean	37.6	30.7+	34.3+	35.8
		SEM	.176	.755	.699	.783
10.0	CWS	Mean	37.2	28.6+	32.7+*	35.0
		SEM	.254	1.22	1.08	.602

Significantly higher (Dunnett comparison, $p<.05$) than 0/No Swim Condition (+) or lower than the 0/CWS Condition (*).

BL=baseline
SEM=standard error

Table 6
The effect of additional doses of scopolamine and methylscopolamine pretreatment upon cold-water swim (CWS) hypothermia as measured by core body temperature.

DOSE (mg/kg)	CONDITION		POST-SWIM (min) BL	30	60	120
Control						
0	CWS (n=6)	Mean	36.7	28.8+	32.4+	35.1
		SEM	.161	1.07	1.05	.448
Scopolamine						
0.001	CWS (n=6)	Mean	36.7	26.8+	31.7+	35.3+
		SEM	.280	.317	.672	.523
0.05	CWS (n=6)	Mean	37.0	27.9+	32.3	34.5+
		SEM	.343	.767	.289	.284
0.5	CWS (n=6)	Mean	35.4	28.0+	31.9+	33.9+
		SEM	1.88	.611	.743	.642
Methylscopolamine						
0.01	CWS (n=6)	Mean	36.7	27.8+	31.9+	34.6+
		SEM	.249	1.11	.762	.645
0.1	CWS (n=6)	Mean	36.7	28.9+	33.3+	35.3
		SEM	.072	.782	.535	.431

Significantly higher (Dunnett comparison, $p<.05$) than 0/No Swim Condition (+) or lower than the 0/CWS Condition (*).

BL=baseline
SEM=standard error

some doses of scopolamine and methylscopolamine potentiated the magnitude and duration of CWS hypothermia. To assess whether alterations of one response measure (analgesia) were related to alterations in a second response measure (hypothermia), a series of correlations (Pearson r) were performed between CWS hypothermia and CWS analgesia on the tail-flick and jump tests. Significant correlations were observed between CWS hypothermia and CWS analgesia on the tail-flick test following pretreatment with scopolamine (Ø.Ø1, Ø.1, 1.Ø and 1Ø.Ø mg/kg) and methylscopolamine (1.Ø and 1Ø.Ø mg/kg), 3Ø min after the swim ($r(6)=.926$, $p<.Ø1$). Following pretreatment with scopolamine (Ø.ØØ1, Ø.Ø5, Ø.5 mg/kg) and methylscopolamine (Ø.Ø1 and Ø.1 mg/kg), significant correlations were observed 3Ø min after the swim ($r(4)=.983$, $p<.Ø1$), 6Ø min after the swim ($r(4)=.956$, $p<.Ø1$) and 12Ø min after the swim ($r(4)=.898$, $p<.Ø2$). These data indicate the potentiations in CWS analgesia observed on the tail-flick test are related to the potentiating hypothermic effects of scopolamine and methylscopolamine pretreatment. This is in contrast to the lack of significant correlations between CWS hypothermia and CWS analgesia on the jump test following scopolamine and methylscopolamine pretreatment.

Experiment 1B: Muscarinic Receptor Antagonists and 2DG Analgesia

Method

Sixty-four rats matched for baseline jump thresholds were assigned to equal groups of 8 rats each: vehicle, scopolamine at doses of 0.01, 0.1, 1.0 and 10.0 mg/kg and methylscopolamine at doses of 1.0 and 10.0 mg/kg. Following assessment of baseline tail-flick latencies and jump thresholds, each rat received their respective drug treatment (e.g. scopolamine or methylscopolamine), 5 min prior to a 600 mg/kg dose of 2DG administered in two successive injections (300 mg/ml normal saline/kg body weight, ip). An additional group of 8 animals received only vehicle injections. Tail-flick latencies and jump thresholds were assessed at 30, 60 and 120 min after the last injection.

After seven to ten days, all rats were reassigned to one of the eight treatments and matched according to their body weights. Each rat received their respective drug treatment 5 min prior to a 600 mg/kg dose of 2DG administered in two successive injections. Food intake was measured 6 hr after the last injection.

Results

2DG Analgesia (Jump Thresholds): Table 7 presents the

Table 7
Scopolamine and methylscopolamine effects upon 2DG analgesia as measured by the jump test (mA).

DOSE (mg/kg)	CONDITION		POST-2DG (min)			
			BL	30	60	120
Control						
0	Vehicle	Mean	.287	.294	.320	.289
		SEM	.020	.013	.009	.014
0	2DG	Mean	.290	.438+	.437+	.354
		SEM	.021	.036	.042	.036
Scopolamine						
0.01	2DG	Mean	.281	.415+	.424+	.374
		SEM	.021	.060	.029	.033
0.1	2DG	Mean	.307	.447+	.408+	.365
		SEM	.011	.038	.033	.028
1.0	2DG	Mean	.305	.533+*	.444+	.397+
		SEM	.020	.049	.056	.032
10.0	2DG	Mean	.307	.409+	.431+	.373
		SEM	.014	.026	.046	.306
Methylscopolamine						
1.0	2DG	Mean	.294	.466+	.444+	.444+*
		SEM	.022	.068	.033	.035
10.0	2DG	Mean	.302	.484+	.457+	.494+*
		SEM	.014	.045	.041	.042

Significantly higher (Dunnett comparison, $p<.05$) than 0/No Swim Condition (+) or lower than the 0/CWS Condition (*).

BL=baseline
SEM=standard error

significant differences in jump thresholds following 2DG among groups ($F(7,56)=2.40$, $p<.032$), across test times ($F(3,168)=46.54$, $p<.001$) and for the interaction between groups and test times ($F(21,168)=1.82$, $p<.021$). Vehicle-treated rats displayed 2DG analgesia on the jump test for up to 60 min after the injection. Rats pretreated with all doses of scopolamine displayed analgesia for up to 60 min. The 1.0 mg/kg dose of scopolamine and all the methylscopolamine-treated rats displayed analgesia for up to 120 min after the 2DG injection. These doses of methylscopolamine also significantly potentiated the magnitude of the 2DG analgesia. Thus, methylscopolamine pretreatment appeared to prolong the duration of 2DG analgesia on the jump test. These results are in marked contrast to the reduction of CWS analgesia following scopolamine and methylscopolamine pretreatment on the jump test.

2DG Analgesia (Tail-Flick Latencies): Table 8 presents the significant differences observed following 2DG on the tail-flick test among groups ($F(7,56)=2.47$, $p<.0258$), across test times ($F(3,168)=19.04$, $p<.0001$) and for the interaction between groups and test times ($F(21,168)=1.92$, $p<.013$). Vehicle-treated rats displayed analgesia on the tail-flick test 60 min and 120 min after the 2DG injection. In contrast, all groups of rats except

Table 8
Scopolamine and methylscopolamine effects upon 2DG analgesia as measured by the tail-flick test (sec).

DOSE (mg/kg)	CONDITION		POST-2DG (min)			
			BL	30	60	120
Control						
0	Vehicle	Mean	2.39	2.18	2.21	2.23
		SEM	.183	.141	.110	.138
0	2DG	Mean	2.41	3.02	3.68+	3.41+
		SEM	.112	.546	.424	.344
Scopolamine						
0.01	2DG	Mean	2.17	3.20	2.91	2.55
		SEM	.112	.536	.396	.158
0.1	2DG	Mean	2.26	3.81+	4.36+	3.48+
		SEM	.160	.522	.451	.216
1.0	2DG	Mean	2.34	4.47+*	3.44+	2.97+
		SEM	.154	.514	.468	.268
10.0	2DG	Mean	2.58	3.82+	3.12	3.02
		SEM	.063	.778	.572	.307
Methylscopolamine						
1.0	2DG	Mean	2.52	4.84+*	3.60+	3.05+
		SEM	.166	.518	.354	.335
10.0	2DG	Mean	2.59	3.10	3.58+	3.15
		SEM	.166	.536	.708	.441

Significantly higher (Dunnett comparison, $p<.05$) than either 0/Vehicle Condition (+) or 0/2DG Condition (*).

BL=baseline
SEM=standard error

the lowest dose of scopolamine (Ø.Ø1 mg/kg) and the highest dose of methylscopolamine (1Ø.Ø mg/kg) displayed 2DG analgesia at 3Ø min. Rats treated with all but the Ø.1 mg/kg dose of scopolamine and the 1.Ø mg/kg dose of methylscopolamine failed to display analgesia at 12Ø min. The latter two doses of scopolamine and methylscopolamine elicited analgesia across the entire time course. Pre-treatment with the 1.Ø mg/kg dose of either scopolamine or methylscopolamine significantly potentiated 2DG analgesia 3Ø min after injection while the highest dose of either scopolamine or methylscopolamine (1Ø.Ø mg/kg) failed to exert any appreciable effect. The lack of any clear consistent pattern by muscarinic receptor antagonists upon 2DG analgesia on the tail-flick test precludes any definitive statement concerning mechanism of action.

2DG Hyperphagia: Table 9 presents the significant dose-dependent reduction in 2DG hyperphagia following scopolamine and methylscopolamine, ($F(7,56)=12.61$, $p<.ØØØ1$). Vehicle-treated rats demonstrated significant increases in food intake following 2DG relative to unstressed rats. Similarly, rats pretreated with low doses of scopolamine (Ø.Ø1 and Ø.1 mg/kg) displayed 2DG hyperphagia comprable to that of the vehicle-treated animals. In contrast, higher doses of either scopolamine (1.Ø and 1Ø.Ø mg/kg) or methylscopolamine (1.Ø and 1Ø.Ø mg/kg)

Table 9
Scopolamine and methylscopolamine effects upon 2DG hyperphagia as measured by food intake (g).

DOSE (mg/kg)			INTAKE (g)
Control			
Ø	Vehicle	Mean	4.ØØ
		SEM	.622
Ø	2DG	Mean	9.97+
		SEM	.659
Scopolamine			
Ø.Ø1	2DG	Mean	1Ø.11+
		SEM	1.56
Ø.1	2DG	Mean	8.99+
		SEM	.594
1.Ø	2DG	Mean	6.14
		SEM	.864
1Ø.Ø	2DG	Mean	4.17
		SEM	.669
Methylscopolamine			
1.Ø	2DG	Mean	5.5Ø
		SEM	.962
1Ø.Ø	2DG	Mean	3.Ø2
		SEM	.677

Significantly higher (Dunnett comparison, $p<.Ø5$) than Ø/Vehicle Condition (+).

SEM=standard error

eliminated 2DG hyperphagia and elicited food intakes similar to that of unstressed control rats.

Experiment 1C: Muscarinic Receptor Antagonism and FPS/HPS Analgesia

Method

Sixty-four rats matched for baseline tail-flick latencies were assigned to equal groups of eight rats each: vehicle, scopolamine at doses of 0.01, 0.1, 1.0 mg/kg and 10.0 mg/kg and methylscopolamine at doses of 1.0 and 10.0 mg/kg. All rats were tested in three different shock conditions (FPS, no shock and HPS). Following measurement of pretreatment tail-flick latencies which consisted of the average of three trials at 10 sec intervals, each rat received their appropriate drug treatment. Five minutes later, they were exposed to FPS. An additional group of eight animals were not exposed to footshock. Tail-flick latencies were assessed 0, 1, 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 min after shock. Six to nine days later, each animal received the same treatment except that no shock was delivered. Following another six to nine day interval, rats were treated identically with the exception that HPS was administered in lieu of FPS.

Results

FPS Analgesia: Table 4 (in appendix) presents the significant differences in tail-flick latencies following FPS across test times ($F(14,184)=34.57$, $p<.0001$) and for

the interaction between groups and test times ($F(14,784)=1.54$, $p<.0012$). Vehicle-pretreated rats displayed FPS analgesia only when tested at 0 and 1 min after FPS. The two lowest doses (0.01 and 0.1 mg/kg) of scopolamine extended FPS analgesia for up to 8 min while the 0.1 and 1.0 mg/kg doses of scopolamine potentiated the analgesia at 1 and 2 min after FPS. While the 1.0 and 10.0 mg/kg doses of scopolamine failed to extend FPS analgesia, the same doses of methylscopolamine elicited analgesia at 6 min after FPS. These data indicate that low doses of scopolamine tended to increase the duration of FPS while higher doses of both scopolamine and methylscopolamine had little effect upon this form of analgesia.

No Shock Condition: While significant differences were observed across test times ($F(14,784)=4.07$, $p<.0001$), the main condition variable or any interactions were not found to be significant. Thus, mere re-exposure to the shock enviroment in the absence of shock was not found to produce analgesia.

HPS Analgesia: Table 5 (in appendix) presents the significant differences in tail-flick latencies following HPS among groups ($F(7,56)=5.52$, $p<.0001$), across test times ($F(14,784)=41.97$, $p<.0000$) and for the interaction between groups and test times ($F(98,784)=2.06$, $p<.0001$).

Vehicle-treated rats displayed analgesia for up to 10 min following HPS. HPS analgesia was present in rats pre-treated with scopolamine at doses of 0.01 mg/kg (16 min), 0.1 mg/kg (20 min) and 10.0 mg/kg (6 min) and methyl-scopolamine at doses of of 1.0 mg/kg (8 min) and 10.0 mg/kg (16 min). The 0.1 mg/kg (14 min) and 1.0 mg/kg (16 min) doses of scopolamine significantly but transitorily potentiated HPS analgesia. Like FPS analgesia, it appears that muscarinic receptor antagonism had little consistent effect upon HPS analgesia.

Experiment 2: Muscarinic Receptor Antagonists and Pilocarpine Analgesia

Method

Forty-two rats matched for baseline jump thresholds were assigned to equal groups of 6 rats each: vehicle, scopolamine at doses of 0.01, 0.1, 1.0 and 10 mg/kg and methylscopolamine at doses of 1.0 and 10.0 mg/kg. Following baseline tail-flick and jump threshold assessments, each rat received their appropriate drug treatment and 5 min later were administered a 10 mg/kg injection of pilocarpine. Tail-flick and flinch jump thresholds were assessed at 30, 60 and 120 min after pilocarpine treatment.

Results

Pilocarpine Analgesia (Jump Thresholds): Table 10 presents the significant differences in jump thresholds following pilocarpine among groups ($F(7,40)=2.26$, $p<.0489$), across test times ($F(3,120)=15.87$, $p<.0001$) and for the interaction between groups and test times ($F(21,120)=2.56$, $p<.0007$). Vehicle-treated rats displayed pilocarpine analgesia on the jump test at 30 min after injection. While animals pretreated with the lowest dose of scopolamine (0.01 mg/kg) displayed normal pilocarpine analgesia, rats pretreated with the higher doses of

Table 10
Scopolamine and methylscopolamine effects upon pilocarpine (PILO, 10 mg/kg, IP) analgesia as measured by the jump test (mA).

DOSE (mg/kg)	CONDITION		POST-INJECTION (min)			
			BL	30	60	120
Control						
0	Vehicle	Mean	.338	.346	.325	.344
		SEM	.016	.014	.022	.015
0	PILO	Mean	.338	.468+	.432	.373
		SEM	.016	.026	.018	.013
Scopolamine						
0.01	PILO	Mean	.281	.491+	.333	.311
		SEM	.019	.115	.039	.032
0.1	PILO	Mean	.271	.446	.407	.357
		SEM	.022	.028	.032	.040
1.0	PILO	Mean	.285	.337*	.319	.327
		SEM	.027	.042	.045	.034
10.0	PILO	Mean	.311	.250*	.310	.353
		SEM	.024	.019	.025	.029
Methylscopolamine						
1.0	PILO	Mean	.307	.471+	.474+	.469+
		SEM	.020	.057	.057	.053
10.0	PILO	Mean	.313	.426	.422	.399
		SEM	.027	.057	.046	.043

Significantly higher (Dunnett comparison, $p<.05$) than the 0/Vehicle condition (+) or lower than the 0/PILO condition (*).

BL=baseline
SEM=standard error

scopolamine (1.0 and 10.0 mg/kg) failed to exhibit an analgesic effect following pilocarpine. Indeed, the higher doses of scopolamine significantly reduced jump thresholds relative to vehicle pretreatment. Rats pretreated with the 1.0 mg/kg dose of methylscopolamine continued to display pilocarpine analgesia across the time course while the 10 mg/kg dose of methylscopolamine eliminated the analgesic effect of pilocarpine. Figure 2 illustrates the degree of pilocarpine analgesia in each experimental condition as a percent increase above the jump thresholds of vehicle-treament. These data indicate that scopolamine, but not methylscopolamine, produce a dose-dependent decrease in pilocarpine analgesia on the jump test. In contrast, methylscopolamine-treated rats (1.0 mg/kg) prolonged the analgesic effect in comparison to the vehicle-treated rats. This corresponds with a proposed central action for pilocarpine analgesia and differs from the reductions observed for CWS analgesia (Figure 1).

Pilocarpine Analgesia (Tail-Flick Latencies): Table 11 presents the significant differences in tail-flick latencies following pilocarpine among groups ($F(7,40)=6.26$, $p<.0001$) and test times ($F(21,120)=3.83$, $p<.0001$). Vehicle-pretreated rats displayed pilocarpine analgesia on the tail-flick test for up to 120 min. Rats

Figure 2. Percent increase in jump thresholds (mA) at 30 and 60 min following pilocarpine (10.0 mg/kg, IP). Groups of six rats each received either vehicle (0), scopolamine (S) at doses of 0.01, 0.1, 1.0 or 10.0 mg/kg (IP) or methylscopolamine (M) at doses of 1.0 or 10.0 mg/kg (IP) 5 min before pilocarpine pretreatment. The values were calculated as the percentage above the jump threshold of the vehicle-treated and unstressed rats (see Table 9). In contrast to CWS analgesia, pilocarpine analgesia was decreased in a dose-dependent manner by S but not M pretreatment. Significant differences form the 0/PILO condition (*) or the 0/vehicle condition (✱).

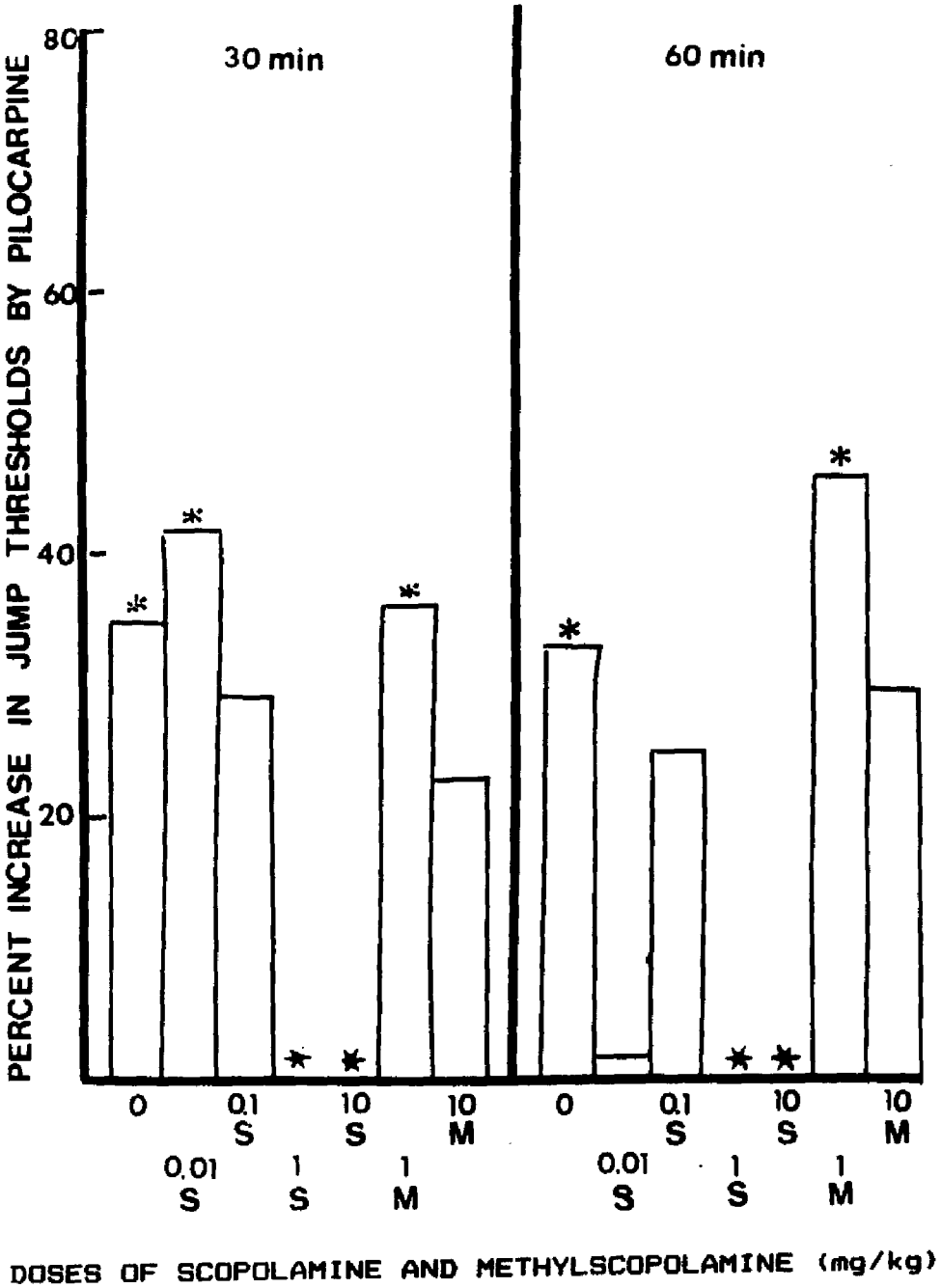
30 min
60 min
PERCENT INCREASE IN JUMP THRESHOLDS BY PILOCARPINE
80
60
40
20
0
0.01 S
0.1 S
1 S
10 S
1 M
10 M
DOSES OF SCOPOLAMINE AND METHYLSCOPOLAMINE (mg/kg)

Table 11. Scopolamine and methylscopolamine effects upon pilocarpine (PILO, 10 mg/kg, IP) analgesia as measured by the tail-flick test (sec).

DOSE (mg/kg)	CONDITION		POST-INJECTION (min)			
			BL	30	60	120
Control						
0	Vehicle	Mean	2.64	2.43	2.66	2.82
		SEM	.267	.338	.200	.357
0	PILO	Mean	2.63	5.53+	5.08+	4.00+
		SEM	.267	.363	.246	.208
Scopolamine						
0.01	PILO	Mean	2.95	5.10+	4.23+	3.70
		SEM	.231	.431	.312	.208
0.	PILO	Mean	2.87	4.87+	4.06+	3.74
		SEM	.145	.345	.567	.239
1.0	PILO	Mean	.2.93	2.99*	3.06*	3.27
		SEM	.182	.223	.230	.272
10.0	PILO	Mean	2.99	3.40*	2.90*	3.50
		SEM	.165	.313	.222	.175
Methylscopolamine						
1.0	PILO	Mean	3.08	4.98+	4.41+	4.13+
		SEM	.285	.409	.437	.438
10.0	PILO	Mean	2.97	4.22+	4.20+	3.96+
		SEM	.216	.575	.408	.342

Significantly higher (Dunnett comparison, $p<.05$) than the 0/Vehicle condition (+) or lower than the 0/PILO condition (*).

BL=baseline
SEM=standard error

pretreated with the lower doses of scopolamine (Ø.Ø1 and Ø.1 mg/kg) displayed pilocarpine analagesia for up to 6Ø min. In contrast, the higher doses of scopolamine (1.Ø and 1Ø.Ø mg/kg) completely eliminated the analgesic effect of pilocarpine. Rats pretreated with methylscopolamine (1.Ø and 1Ø.Ø mg/kg) displayed pilocarpine analgesia across the time course. These data indicate that scopolamine, but not methylscopolamine, reduces pilocarpine analgesia in a dose-related manner and contrasts with the observed potentiation on the tail-flick test following CWS and 2DG analgesia.

Experiment 3: Muscarinic Receptor Antagonists and Basal Thresholds

Method

Forty-two rats matched for baseline jump thresholds were assigned to equal groups of 8 rats each: vehicle, scopolamine at doses of 0.01, 0.1, 1.0 and 10.0 mg/kg and methylscopolamine at doses of 1.0 and 10.0 mg/kg. Following determination of baseline core body temperatures, tail-flick latencies and jump thresholds, each rat received their respective drug injection. These measures were reassessed 30, 60 and 120 min later. After 7-10 days, all rats were matched according to their body weight and were reassigned to one of the drug groups. Food intake was then assessed six hrs after injection.

Results

Jump Thresholds: Table 12 presents the effect of scopolamine and methylscopolamine on basal jump thresholds. A significant interaction was observed between groups and test times ($F(18,105)=p<.0017$). Rats pretreated with vehicle and all doses of scopolamine (excluding the 1.0 mg/kg dose at 30 min and the 10.0 mg/kg dose at 60 min) and methylscopolamine displayed consistent jump thresholds for up to 120 min and failed to differ from each other. These data taken together with the data

Table 12
Scopolamine and methylscopolamine effects upon basal jump thresholds (mA).

DOSE (mg/kg)		POST-INJECTION (min)			
		BL	30	60	120
Control					
0	Mean	.280	.281	.264	.294
	SEM	.026	.020	.016	.018
Scopolamine					
0.01	Mean	.334	.315	.343	.306
	SEM	.033	.029	.032	.032
0.1	Mean	.268	.267+	.343	.332
	SEM	.017	.022	.027	.026
1.0	Mean	.295	.226+	.242	.264
	SEM	.021	.029	.042	.031
10.0	Mean	.278	.233	.221+	.263
	SEM	.024	.025	.028	.027
Methylscopolamine					
1.0	Mean	.285	.329	.322	.333
	SEM	.015	.019	.025	.028
10.0	Mean	.274	.325	.301	.303
	SEM	.014	.026	.027	.034

Significantly lower (Dunnett comparison, $p<.05$) than the Vehicle condition (+).

BL = baseline
SEM = standard error

on scopolamine and methylscopolamine action upon CWS, 2DG and pilocarpine analgesia indicates that the drugs had an effect on analgesia and was not the result of baseline shifts in jump thresholds.

Tail-Flick Latencies: Table 13 presents the failure of scopolamine and methylscopolamine to alter basal tail-flick latencies. Significant differences were not observed between groups ($F(6,35)=1.19$) and across test times ($F(30,105)=1.19$). These data indicate scopolamine and methylscopolamine effects upon CWS, 2DG and pilocarpine analgesia were not the result of baseline shifts in basal tail-flick latencies.

Core Body Temperatures: Table 14 presents the significant differences in core body temperatures in scopolamine and methylscopolamine treated rats across test times ($F(3,84)=1.17$, $p<.0004$). Scopolamine and methylscopolamine pretreatment failed to alter basal core body temperatures and indicate that their effectiveness in potentiating CWS hypothermia was not the result of baseline shifts in core body temperature.

Food intake: Table 15 presents the significant differences in food intake among groups ($F(6,35)=5.47$, $p<.0004$). While low doses of scopolamine (0.01 and 0.1

Table 13
Scopolamine and methylscopolamine effects upon basal tail-flick latencies (sec).

DOSE (mg/kg)		POST-INJECTION (min)			
		BL	30	60	120
Control					
0	Mean	3.09	3.20	2.92	2.84
	SEM	.208	.358	.181	.334
Scopolamine					
0.01	Mean	3.21	3.78	3.50	3.55+
	SEM	.262	.297	.244	.280
0.1	Mean	3.13	3.04	3.23	3.07
	SEM	.237	.125	.159	.100
1.0	Mean	2.91	2.66	2.59	3.15
	SEM	.196	.294	.266	.241
10.0	Mean	3.32	2.89	3.08	3.72+
	SEM	.219	.258	.355	.331
Methylscopolamine					
1.0	Mean	3.45	3.46	3.50	3.77+
	SEM	.460	.330	.281	.578
10.0	Mean	3.20	3.49	3.54	3.34
	SEM	.308	.278	.287	.379

Significantly higher (Dunnett comparison, $p<.05$) than the Vehicle condition (+).

BL=baseline
SEM=standard error

Table 14
Scopolamine and methylscopolamine effects upon basal core body temperature (°C).

DOSE (mg/kg)		POST-INJECTION (min)			
		BL	30	60	120
Control					
0	Mean	36.74	36.88	36.70	36.84
	SEM	.425	.347	.239	.223
Scopolamine					
0.01	Mean	36.26	36.20	36.36	36.02+
	SEM	.460	.303	.136	.363
0.1	Mean	36.38	36.46	36.30	36.44
	SEM	.120	.163	.141	.269
1.0	Mean	37.76	37.38	37.12	36.52
	SEM	.664	.649	.486	.558
10.0	Mean	36.72	36.48	36.26	36.52
	SEM	.321	.563	.306	.332
Methylscopolamine					
1.0	Mean	36.52	36.32	36.28	36.08
	SEM	.218	.128	.193	.275
10.0	Mean	37.04	36.44	36.54	36.24
	SEM	.353	.344	.180	.267

Significantly higher or lower (Dunnett comparison, $p<.05$) than the Vehicle condition (+).

BL=baseline
SEM=standard error

Table 15
Scopolamine and methylscopolamine effects upon basal feeding (g).

DOSE (mg/kg)		INTAKE (g)
Control		
Vehicle	Mean	3.93
	SEM	.896
Scopolamine		
Ø.Ø1	Mean	3.Ø2
	SEM	.771
Ø.1	Mean	2.37
	SEM	.427
1.Ø	Mean	.982+
	SEM	.292
1Ø.Ø	Mean	1.Ø8+
	SEM	.2Ø9
Methylscopolamine		
1.Ø	Mean	.931+
	SEM	.379
1Ø.Ø	Mean	.987+
	SEM	.2Ø1

Significantly lower (Dunnett comparison, $p<.05$) than the Ø mg/kg dose (vehicle) condition (+).

SEM=standard error

mg/kg) elicited similar intake to vehicle pretreatment, rats pretreated with higher doses of scopolamine and methylscopolamine (1.Ø and 1Ø.Ø mg/kg) displayed significant decreases in food intake. This dose-dependent suppression of food intake is similar to the effects of scopolamine and methylscopolamine pretreatment upon 2DG hyperphagia. Correlations were performed to determine whether the dose-dependent decreases in 2DG hyperphagia following pretreatment with scopolamine and methylscopolamine were associated with the corresponding dose-dependent decreases in basal food intake. A significant correlation between reductions in 2DG hyperphagia and basal food intake following pretreatment of scopolamine and methylscopolamine was observed ($r(35)=.897$, $p<.ØØØ1$).

Experiment 4: Muscarinic Receptor Antagoinists and Opioid Analgesia

Method

Eight cannulated rats, matched post-operatively for baseline jump thresholds, received three conditions at one week intervals in a counterbalanced order: vehicle (1 ml normal saline kg body weight, IP) / vehicle (5 ug normal saline, ICV), vehicle (1 ml normal saline kg body weight) / DADL (40 ug, ICV), and scopolamine (10 mg/kg, IP) / DADL (40 ug, ICV). A second and third group of eight rats each were exposed to an identical paradigm except that either BEND (1 ug, ICV) or morphine (5 mg/kg, SC) was administered in lieu of DADL. The third group of rats also received naloxone (5 mg/kg, SC) / morphine (5 mg/kg, SC). A 5 min delay seperated the two injections in all conditions. Jump thresholds were assessed at 15, 30, 45 and 60 min following the last injection in the first and second groups and at 30, 60, 90 and 120 min following the last injection in the third group.

Results

Scopolamine and DADL Analgesia: Table 16 presents the effect of scopolamine pretreatment upon DADL analgesia on the jump test. Significant differences were observed among groups ($F(4,28)=6.84$, $p<.0006$) and for the interac-

Table 16
The effect of scopolamine (SCOP, 10 mg/kg, IP) pretreatment upon D-ala-D-leu enkephalin (DADL, 40 ug/ul, ICV) analgesia as measured by the jump test (MA).

CONDITION		POST-INJECTION (min)				
		PRE	15	30	45	60
Vehicle/ Vehicle	Mean	.327	.327	.339	.316	.317
	SEM	.019	.020	.014	.016	.012
Vehicle/ DADL	Mean	.291	.408+	.394+	.302	.300
	SEM	.009	.045	.023	.011	.012
SCOP/ DADL	Mean	.340	.439+	.452+	.416+*	.397+*
	SEM	.012	.036	.046	.043	.037

Significantly higher (Dunnett comparison, $p<.05$) than the Vehicle/Vehicle Condition (+) or the Vehicle/DADL condition (*).

PRE=preinjection
SEM=standard error

tion between groups and test times ($F(8,56)=2.37$, $p<.029$). DADL significantly increased jump thresholds for up to 30 min after injection. Scopolamine pretreatment significantly potentiated the duration of DADL analgesia for the entire 60 min time course.

Scopolamine and BEND Analgesia: Table 17 presents the effect of scopolamine upon BEND analgesia on the jump test. Significant differences were observed among groups ($F(2,14)=26.76$, $p<.0001$), across test times ($F(4,28)=8.69$, $p<.0001$) and for the interaction between groups and test times ($F(8,56)=3.97$, $p<.0009$). Both BEND and scopolamine paired with BEND produced analgesia across the 60 min time course. Scopolamine pretreatment failed to alter the magnitude of BEND analgesia.

Scopolamine and Morphine Analgesia: Table 18 presents the effect of scopolamine pretreatment on morphine analgesia on the jump test. Significant differences were observed among groups ($F(3,21)=16.78$, $p<.0001$), across test times ($F(4,28)=26.88$, $p<.0001$) and for the interaction between groups and test times ($F(2,84)=9.43$, $p<.0001$). Both morphine and scopolamine paired with morphine produced analgesia across the 120 min time course. Scopolamine pretreatment signficantly potentiated morphine analgesia for up to 90 min after injection. Naloxone

Table 17
The effect of scopolamine (SCOP, 10 mg/kg, IP) pre-treatment upon beta-endorphin (BEND, 1 ug/ul, ICV) analgesia as measured by the jump test (MA).

CONDITION		POST-INJECTION (min)				
		PRE	15	30	45	60
Vehicle/ Vehicle	Mean	.273	.262	.260	.287	.284
	SEM	.008	.015	.009	.011	.006
Vehicle/ B-END	Mean	.315	.393+	.401+	.362+	.330+
	SEM	.016	.011	.022	.028	.020
SCOP/ B-END	Mean	.292	.361+	.384+	.373+	.350+
	SEM	.012	.017	.027	.025	.029

Significantly higher (Dunnett comparison, $p<.05$) than the Vehicle/Vehicle Condition (+) or the Vehicle/B-END condition (*).

PRE=preinjection
SEM=standard error

Table 18
The effect of scopolamine (SCOP, 10 mg/kg, IP) and naloxone (NAL, 5 mg/kg, SC) pretreatment upon morphine (MOR, 5 mg/kg, SC) analgesia as measured by the jump test (mA).

CONDITION		POST-INJECTION (min)				
		PRE	30	60	90	120
Vehicle/ Vehicle	Mean	.279	.292	.310	.306	.306
	SEM	.011	.013	.011	.012	.018
Vehicle/ Morphine	Mean	.312	.598+	.564+	.470+	.412+
	SEM	.024	.045	.036	.025	.024
SCOP/ Morphine	Mean	.306	.704+*	.708+*	.644+*	.456+
	SEM	.020	.079	.093	.102	.089
NAL/ Morphine	Mean	.301	.310*	.300*	.315*	.304*
	SEM	.013	.013	.009	.011	.010

Significantly higher (Dunnett comparison, $p<.05$) than the Vehicle/Vehicle condition (+) or the Vehicle/Morphine condition (*).

PRE=preinjection
SEM=standard error

eliminated morphine analgesia across the time course. These data indicate the differential effects of the muscarinic receptor anatagonist, scopolamine upon opioid analgesia by potentiating morphine and DADL analgesia while having no affect on BEND analgesia.

Discussion

The results of the present study are summarized in Table 6 (in appendix). In general, pretreatment with scopolamine and methylscopolamine, two muscarinic receptor antagonists, differentially affect stress-induced analgesia, depending upon the particular stressor and pain test employed. Scopolamine also differentially affected opioid analgesia depending upon which opioid or opiate was administered. These effects can be examined directly by comparing the greater potency of the centrally active (scopolamine) with the peripherally active (methylscopolamine) muscarinic receptor antagonist and their ability to block analgesia induced by a muscarinic receptor agonist, pilocarpine, in a dose dependent manner. Furthermore, the observed changes in the analgesic responses to stress and opioids by muscarinic receptor antagonism cannot be attributed to baseline shifts in reactivity.

In assessing the role of the muscarinic receptor in stress-induced analgesia, four stressors with different physiological and pharmacological analgesic profiles were employed: CWS (nonopioid-neurohormonal), 2DG (opioid-neurohormonal), FPS (opioid-neural) and HPS (nonopioid-neural). Of these, scopolamine and methylscopolamine exerted the most profound effects upon CWS analgesia in a test-specific manner. Both muscarinic antagonists at-

tenuated CWS analgesia on the jump test and potentiated CWS analgesia on the tail-flick test. While vehicle treated rats displayed CWS analgesia on the jump test for up to 120 min following the swim, scopolamine and methyl-scopolamine pretreatment reduced CWS analgesia 30 min following the swim and significantly reduced this response at 60 min after the swim. Since a clear dose response relationship failed to emerge for the latter effect, it would appear that the strongest statement that can be made is that the muscarinic receptor is important for the maintainance of CWS analgesia on the jump test. The reductions observed for CWS and pilocarpine analgesia on the jump test following muscarinic antagonism appear to differ in that the latter response displays a clear dose response effect and is influenced more by scopolamine which has both central and peripheral actions than by methylscopolamine which has only peripheral action. This is in keeping with suggestions of a central mode of action for pilocarpine analgesia Houser, 1976; Houser & Van Hart, 1974). In contrast, the similar actions of scopolamine and methylscopolamine upon CWS analgesia on the jump test suggest mediation through a peripheral cholinergic mechanism. Such a supposistion coincides with data indicating a neurohormonal substrate for CWS analgesia as measured by the jump test. The hypothalamo-hypophysial axis is implicated since destruction of either the medial-basal

hypothalamus (Badillo-Martinez et al., 1984; Bodnar et al., 1980a) or the pituitary gland (Bodnar et al., 1979a) decreases CWS analgesia. This neurohormonal component appears to be mediated through the adrenocortical system since a) posterior lobectomy fails to affect CWS analgesia (Glusman, Bodnar, Kelly, Sirio, Stern & Zimmerman, 1979), b) adrenalectomy (Glusman et al., 1980; Marek et al., 1982) but not adrenal demedullation (Bodnar et al., 1982) potentiates CWS analgesia and c) corticosterioid activation and inhibition, respectively, decrease and increase CWS analgesia (Marek et al., 1982; Mousa et al., 1981, 1983). In this regard, cholinesterase inhibitors increase corticosterone (Kolta & Soliman, 1981) and adrenocorticotrophic hormone (Civen, Loeb, Wishow, Wolfson & Manin, 1980) as well as affecting the hypothalamo-pituitary axis (Ruiz, deGallarita, Fanjul & Meites, 1981).

While scopolamine and methylscopolamine pretreatment decrease CWS analgesia on the jump test, they have the opposite effect on the tail-flick test and appear to produce small transient potentiations. The appearance of test-specific effects can be the result of several factors. First, the analgesic time course following CWS varies as a function of the pain test with optimal analgesia occurring at 30 min for the jump test and at 30-180 min for the tail-flick test (Bodnar et al., 1978d). Differences between the two tests have also been observed

following repeated exposure to CWS. Chronic exposure to CWS for 14 consecutive days eliminates CWS analgesia on the jump test while only reducing CWS analgesia on the tail-flick test (Bodnar et al., 1978d; Bodnar and Komisurak, 1984). However, a main factor for this discrepency between pain tests may be attributed to the possible influence of CWS hypothermia upon CWS analgesia on the tail-flick test. The potentiation in CWS analgesia on the tail-flick test and the potentiation in CWS hypothermia by muscarinic receptor antagonism were signficantly correlated. This also parallels the proximal to distal recovery of vasodilation with the tail being one of the last structures to experience normal thermoregulation following CWS. Therefore, the data suggest that the potentiation of CWS analgesia on the tail-flick test by muscarinic receptor antagonism is an epiphenomenon of similar changes in CWS hypothermia and not the result of specific changes in pain inhibition. In contrast, the reduction in CWS analgesia on the jump test by scopolamine and methylscopolamine did not correlate with hypothermic changes providing further evidence that the former effects were the result of alterations in an endogenous analgesic system.

Scopolamine and methylscopolamine pretreatment differentially altered CWS and 2DG analgesia on the jump test. Despite the development of complete and reciprocal

analgesic cross tolerance (Spiaggia et al., 1979) and their similar modulation by the dopaminergic system (Bodnar et al., 1980b; Bodnar and Nicotera, 1982) 2DG and CWS analgesia often display opposite effects. Both muscarinic receptor anatagonists correspondingly reduced CWS analgesia and potentiated 2DG analgesia suggesting that peripheral cholinergic mechanisms may modulate these responses. In addition, since the potentiation of 2DG analgesia by scopolamine and methylscopolamine occurred at 120 min after the stressor, it would appear that cholinergic modulation involves the maintainance rather than the initiation of the response. This dichotomy between CWS and 2DG analgesia also occurs following manipulations of the hypothalamo-hypophysial axis in that both medial-basal hypothalamic damage (Badillo-Martinez et al., 1984) and hypophysectomy (Bodnar et al., 1979a) potentiate 2DG analgesia. Further, while pretreatment with the noradrenergic reuptake inhibitor, desipramine, potentiates CWS analgesia, it reduces 2DG analgesia (Bodnar et al., 1985, 1986).

Our laboratory has proposed a mechanism by which a given physiological or pharmacological manipulation is able to potentiate one analgesic response and reduce another. The process of collateral inhibition hypothesis postulates that the two manipulations are activating distinct pain inhibitory systems such that excitation of one

results in the inhibition of the other (Kirchgessner et al., 1982). That is, activation of one system by a given stressor (e.g. CWS) inhibits the activation of a second system, precluding simultaneous activation of all systems during stress and thereby allowing the alternative (and less relevant) systems to be held in reserve. If the cholinergic system is involved in the maintainance of the pain inhibitory system activated by CWS and also inhibits that system by 2DG, it would be expected that cholinergic antagonism would reduce CWS analgesia directly and potentiate 2DG analgesia through disinhibition. This relationship has also been observed for CWS and morphine analgesia. Anti-enkephalinases potentiate morphine analgesia while reducing CWS analgesia (Bodnar et al., 1982), while irreversible opiate receptor antagonists reduce morphine analgesia and potentiate CWS (Kirchgessner et al., 1982). Since 2DG analgesia is partially mediated by an opiate mechanism (Bodnar et al., 1979b; Spiaggia et al., 1979) and since both 2DG and morphine analgesia are potentiated by scopolamine, this collateral inhibition may be working through the opiate component of 2DG analgesia. Finally, this hypothesis may explain the opposite effects upon CWS and 2DG analgesia following the aforementioned medial-basal hypothalamic damage and hypophysectomy.

While the effects upon 2DG analgesia following muscarinic antagonism appeared not to be the result of

effects of scopolamine and methylscopolamine upon basal pain thresholds, a different pattern emerged for 2DG hyperphagia. The reductions induced by scopolamine and methylscopolamine upon basal food intake and 2DG hyperphagia showed strong significant correlations suggesting that they are mediated through the same mechanism. Since scopolamine and methylscopolamine were equally effective in reducing both responses, it would appear that these effects could be most parsimoniously attributed to blockade of peripheral parasympathetic responses. This dissociation between the analgesic and hyperphagic response to 2DG is not novel (see review: Bodnar et al., 1986). Indeed, the only instance in which both responses are reduced is in the aging process (Kramer, Sperber & Bodnar, 1985).

The effects of scopolamine and methylscopolamine upon footshock analgesia in the present study again demonstrate the importance of parametric consideration in describing pharmacological and physiological profiles of shock analgesia. In contrast to the inability of muscarinic receptor antagonism to appreciably affect FPS or HPS analesia, in the present study, scopolamine reduced analgesia induced by prolonged, intermittent footshock but not brief, continuous footshock (Lewis et al., 1983). Furthermore, scopolamine signficantly reduced analgesia induced by brief re-exposure to inescapable shock (MacLennan

et al., 1982). It is interesting to note that both of the latter forms of muscarinic-sensitive analgesia also possess hormonal components (Lewis et al., 1980, 1982; MacLennan et al., 1983). However, the possibility that a common linking mechanism can be attributed to the muscarinic effect upon these forms of footshock analgesia and CWS analgesia is questionable given that a) methylscopolamine is active in the latter types, but not in the former types of analgesia and b) opiate antagonism is effective in the former, but not in the latter types of analgesia (see reviews: Bodnar et al., 1984, 1986; Terman et al., 1984).

While low doses of scopolamine were found to transiently potentiate HPS analgesia in the present study, Watkins and co-workers (1984) found that equivalent doses of scopolamine attenuated HPS analgesia. One possible reason for this discrepency may be the injection-test interval between the two studies, 30 min (Watkins et al., 1984) vs 5 min (the present study). Second, the magnitude and duration of HPS analgesia in the present study was less than that reported previously (Watkins et al. 1984) despite the fact that identical shock parameters were used. Other laboratories (Cannon et al., 1982; Terman et al., 1984; Urca et al., 1985) have demonstrated that relatively small changes in shock parameters can alter the pharmacological profile of the subsequent analgesic re-

sponse. It is conceivable that different levels of shock may have been administered by the different equipment (shock generator, scrambler or test box). Despite the differences between our laboratory and Watkins, it is still clear that muscarinic receptor antagonism exerts differential effects upon stress induced analgesia that varies as a function of the stressor and the pain test. All of these effects appear not to be mediate through central cholinergic mechanisms as they do not display the dose-dependent reduction by scopolamine as observed for pilocarpine analgesia on both pain tests. Furthermore, they do not show the greater efficacy of scopolamine over methylscopolamine in exerting such effects.

Prior research using synergistic interactions suggested that analgesia induced by opiates and cholinomimetics interact. However, subsequent studies questioned this hypothesis since cholinergic or opiate receptor antagonists only partially or failed to attenuate the other form of analgesia (Calcutt & Spence, 1972; Ireson, 1970; Ramabradan & Jacob, 1978; Tobias, 1971). Despite the development of analgesia tolerance by both agents, opiate and cholinergic agonists failed to develop an analgesic cross-tolerance (Howes et al., 1968; Little & Rees, 1974; Pedigo & Dewey, 1981; Pert & Maxey, 1975). Indeed some studies have reported potentiation of opiate analgesia by cholinergic receptor antagonists on the

tail-flick (Lewis et al., 1983) and shock titration (Pert & Maxey, 1975) tests. The ability of scopolamine to potentiate DADL and morphine analgesia, but not BEND analgesia on the jump test is in agreement with the latter studies. This potentiation cannot be attributed to shifts in baseline thresolds by scopolamine nor can they be explained in terms of any nonspecific effect since scopolamine selectively blocked morphine and DADL analgesia but not BEND analgesia. The ability of naloxone to eliminate morphine analgesia confirmed the opiate effect.

Scopolamine's differential effects on opiate and opioid analgesia confirms other evidence that the opiate pain inhibitory system is not a single homogeneous mechanism (e.g. Yaksh, 1981; Yeng and Rudy, 1980a,b). It would appear that different groups of opioid peptides interact with the different opiate receptor subtypes (mu, delta, kappa and epsilon). These opioid peptides and receptors are differentially distributed in spinal and supraspinal structures (Atweh & Kuhar, 1977a,b,c) and may mediate different forms of analgesia. For instance, the mui receptors appear to mediate supraspinal analgesia (Pasternak, 1981; Pasternak et al., 1980, 1981; Zhang & Pasternak, 1981) while delta and kappa mediate spinal analgesia (Schmauss & Yaksh, 1984).

The present research suggests that cholinergic analgesia is not mediated through an opioid pain inhibitory

system. Biochemical studies demonstrate differences in stereoselectivity, pA2 values and the slope of modified Schild plots for morphine antagonists (naloxone and pentazocine). Inhibition of ACh and morphine suggest opiate receptor antagonism varies as a function of whether the analgesia was produced by ACh or morphine. In addition, the anatomical pathways mediating ACh and morphine analgesia appear to be distinct. While opiate antinociception appears to involve the DLF, NRM and PAG (Yaksh & Rudy, 1978; Mayer & Price, 1976; Messing & Lytle, 1977; Gebhart, 1982) this does not appear to be the case for cholinergic analgesia. Microinjections of either ACh into the PAG (Metys et al., 1969) or carbachol into the NRM (Brodie & Proudfit, 1982) produce analgesia. While cells in the NRM are excited by iontophoretically applied ACh and are inhibited by scopolamine, these responses do not affect neurons in the PAG, nor does stimulation of the DLF or PAG affect cells in the NRM which are sensitive to ACh or scopolamine (Couch, 1970; Bradely & Dray, 1974; Behbehani, 1982). Therefore, while ACh acts through the muscarinic receptor as an excitatory neurotransmitter in the NRM, its effects are independent of opioid-mediated PAG stimulation. Furthermore, while opiate receptor density is greatest in the vicinity of the PAG, only a small number of muscarinic receptors and low densities of cholinergic neurons or CAT levels have been detected (Wamsley et al.,

1981; Kuhar & Yamamura, 1976; Kimura, McGeer, Peng, McGeer, 1981; Kuhar et al., 1981). Scopolamine fails to affect local glucose metabolism in either the PAG or ventral brainstem (Weinberg, Greenberg, Waldo, Sylvest & Reivich, 1979). Thus, while some cholinergic analgesia is mediated by some of the same structures (NRM and PAG) which are involved in morphine analgesia, the neuronal pools appear to be independent of one another (Chau & Dewey, 1981). There appears to be an area in the brain in which carbachol (a cholinomimetic) can elicit analgesia while neither morphine nor naloxone exert effects (Katayama et al., 1982, 1984). This area is located adjacent to the dorsolateral brachium conjunctivm and the parabrachial nucleus in the reticular formation and has numerous muscarinic, nicotinic and opiate receptors (Wamsley et al,. 1981; Segal, Dudai & Amsterdam, 1978; Pearson et al., 1980). Lesions placed in the DLF partially block carbachol analgesia elicited from this tegmental area (Hayes et al., 1984). This is not mediated through a direct connection since horseradish peroxidase (HRP) studies indicate that cholinergic neurons of the dorsolateral brachium conjunctivm do not project directly through the DLF (Hayes et al., 1984). Thus, it seems that cholinergic analgesia may be mediated through a polysynaptic pathway including the parabrachial nucleus which possesses reciprocal projections with brainstem nuclei

involved in descending bulbo-spinal pain inhibition as the nucleus raphe dorsalis and magnus (Morgane, Forbes & Pasquier, 1974; Taber-Pierce, Foote & Hobeson, 1976; Beitz, 1982; Bobillier, Seguin, Pettijean, Salvert, Touret & Jouvet, 1976) and the PAG (Sakai, Touret, Salvert, Leger & Jouvet, 1977). In addition, local glucose utilization in the parabrachial nucleus increases following PAG stimulation at intensities associated with analgesia (Beitz & Buggy, 1982).

Having established the existence of a separate cholinergic component of pain inhibition, the link between cholinergic antinociception and other neurotransmitters will be examined. Serotonin has been implicated in the mediation of pain inhibition (see reviews: Mayer & Price, 1976; Messing & Lytle, 1977). ACh and serotonin have been observed to interact such that physostigmine increases serotonin turnover (Reid, 1970; Barbeau, Haubrich & Reid, 1972) while ACh and cholinomimetics stimulate serotonin release (Ascher, Glowinski, Tave & Taxi, 1968) and decrease the serotonin metabolite, 5HIAA during analgesia (Paalzow & Paalzow, 1975). Raphe lesions decrease ACh activity in the cortex (Garau, Mula & Pepeu, 1975). Physostigmine and tremorine induced analgesia are attenuated by reductions of serotonin activity following reserpine (monoaminergic storeage depletor), PCPA (inhibitor of tryptophan hydroxylase), methysergide (serotonin re-

ceptor blocker) and 5,6-dihydroxytrytamine (serotonin neurotoxin) (Koe & Weissman, 1966; Baumgarten, Bjorland, Lachenmeyer, Novin & Stenevi, 1971; Pleuvry & Tobias, 1971; Bhattacharva & Nayak, 1978). In contrast, 5-hydroxytryptophan (serotonin percursor) potentiated physostigmine analgesia (Pleuvry & Tobias, 1971). However, serotonin's involvement in cholinergic analgesia is still in question since oxytremorine induced analgesia was not effected by 5-hydroxytyrtophan, PCPA, alpha-methyltyrosine, l-tryptophan and LSD-25 (Pleuvry & Tobias, 1971; Paalzow & Paalzow, 1975). It should be noted that while serotonin depletion reduces opiate analgesia, serotonin stimulation has the opposite effect and fails to affect CWS analgesia (Bodnar et al., 1981a; Brutus et al., 1979; Messing & Lytle, 1977).

A catecholaminergic component in cholinergic analgesia has been postulated since dopamine and noradrenergic metabolism are affected by cholinergic agonists (Corodi et al., 1967; Nose & Takemoto, 1974). However, the role of catecholamines in ACh antinociception is not firmly established. Increases in catecholamine levels by phenoxybenzamine or L-Dopa pretreatment either increased, decreased, or failed to affect physostigmine and oxotremorine analgesia (Pleuvry & Tobias, 1971; Paalzow & Paalzow, 1975). Decreases in catecholamine activity by either reserpine, AMPT or NE synthesis inhibitors (H44/68 and

diethyldithiocarbamate) either potentiated or reduced physostigmine and oxotremorine analgesia (Paalzow & Paalzow, 1975; Pleuvry & Tobias, 1971).

The previous pharmacological, biochemical and anatomical evidence seem to indicate the presence of a non-opioid cholinergically activated pain inhibitory system. If such a system exists, this alternative cholinergic mechanism should have its own functional significance. The cholinergic system has long been established to be an integral part of learning and memory processes. Increased levels of ACh facilitate learning and memory tasks in animals and humans while lowered ACh levels have been associated with learning and memory impairments (Drachman, 1970; Ferris, 1980; Meyers, 1965; Suits & Isaacson, 1970; White, 1972). The cholinergic system has also been associated with attentional mechanisms since cholinergic receptor blockers increase distractibility and decrease habituation (Carlton & Vogel, 1965). Furthermore, Ach is found in abundance in limbic structures as the hippocampus, amygdala and the septohippocampal pathway (Lewis & Shute, 1965), all of which are associated with learning, memory and attentional processes. In fact, these areas have been observed to be involved in cholinergic analgesia. Low doses of carbachol in the septal nucleus produce analgesia and this effect is eliminated by the cholinergic receptor antagonist atropine (Metys et al., 1969). It is

therefore possible that the cholinergic mediation of analgesia is related to its involvement in learning, memory and attentional processes. Thus, cholinergic agents may alter an animal's attentional processes to a stressor or the memory of the stressful event and may be different than that following analgesia induced by opiates. If changes occur in how the animal perceives the stressful stimulus then the stressful consequences of the stimulus may also be altered. This is highly speculative but further research may amplify the interactions among the previously described sensory-discrimnative, motivational-affective and cognitive aspects of pain (Melzack & Casey, 1967) and the endogenous systems that exert inhibitory control over such responses.

In conclusion, the present studies replicate some previously established findings regarding the cholinergic system and pain inhibition. For instance, the cholinergic agonist, pilocarpine, was found to produce an analgesic state that could be blocked by scopolamine but not methylscopolamine. In addition, it was demonstrated that while scopolamine alone had no affect on basal pain thresholds it did affect analgesia induced by various stressors. Although scopolamine was found to interact with morphine analgeisa, anatomical, biochemical and physiological evidence suggests that the two are mediated by independent pain inhibitory systems. Along these

lines, scopolamine, in contrast to morphine, appears to have a greater affect on the duration of an analgesic response following a stressor as CWS. Thus, it is possible that cholinergic pain inhibition is a secondary system to that of the opiate system. However, the precise mechanism by which acetylcholine is involved in pain is still unclear. Most of scopolamine's affect on stress-induced analgesia can be accounted for by the drugs peripheral activity. Therefore, perhaps if scopolamine were administered centrally, particularly in areas of the septohippocampal pathway, parabrachial nucleus or adjacent to the brachium conjunctivm, in addition to the NRM and PAG, it would have a greater affect on analgesic processes. In addition, as it has already been established that there are more than one type of muscarinic receptors (M1 and M2, see Birdsall, Burgen & Hulme, 1978; Hammer, Berrie, Birdsall, Burgen & Hulme, 1980) perhaps future research employing an M2 receptor anatagonist (as pirenzepine) would be found to have greater effect on stress-induced analgesia or opiate analgesia than scopolamine which has less specific activity at these receptors. Finally, the discovery of a potent analgesic agent without any of the side effects of morphine (as addiction) would be beneficial. To this end, anti-AChEI have been employed clinically to potentiate morphine analgesia at lower doses. However, future research is still necessary to

determine a potent analgesic without any adverse side effects.

APPENDIX

Table 1

The effect of all doses of scopolamine and methyl-scopolamine pretreatment upon cold-water swim (CWS) analgesia as measured by the jump test (mA).

DOSE (mg/kg)	CONDITION		POST-SWIM (min)			
			BL	30	60	120
Control						
0	No Swim	Mean	.306	.281	.305	.312
	(n=8)	SEM	.012	.015	.016	.028
0	CWS	Mean	.291	.463+	.399+	.390+
	(n=8)	SEM	.037	.040	.045	.044
0	CWS	Mean	.335	.421+	.379+	.415+
	(n=6)	SEM	.029	.048	.035	.023
Scopolamine						
0.001	CWS	Mean	.334	.351	.486+	.432+
	(n=6)	SEM	.024	.039	.079	.051
0.01	CWS	Mean	.304	.353*	.365	.313
	(n=8)	SEM	.023	.066	.047	.043
0.05	CWS	Mean	.343	.365	.321	.378
	(n=6)	SEM	.017	.024	.025	.021
0.1	CWS	Mean	.297	.274*	.301*	.308
	(n=8)	SEM	.016	.024	.029	.013
0.5	CWS	Mean	.304	.311	.302	.311
	(n=6)	SEM	.029	.022	.027	.039
1.0	CWS	Mean	.309	.382+	.325	.308
	(n=8)	SEM	.014	.037	.033	.024
10.0	CWS	Mean	.306	.384+	.306	.335
	(n=8)	SEM	.029	.044	.033	.023

Table 1 (continued)

Methylscopolamine						
Ø.Ø1	CWS	Mean	.268	.347	.3Ø4	.372
	(n=6)	SEM	.Ø85	.Ø4Ø	.Ø37	.Ø38
Ø.1	CWS	Mean	.317	.438+	.374	.434+
	(n=6)	SEM	.Ø34	.Ø63	.Ø57	.Ø48
1.Ø	CWS	Mean	.3Ø4	.328*	.333	.346
	(n=8)	SEM	.Ø26	.Ø43	.Ø47	.Ø35
1Ø.Ø	CWS	Mean	.3Ø7	.423+	.345	.331
	(n=8)	SEM	.Ø14	.Ø66	.Ø25	.Ø23

Significantly higher (Dunnett comparison, $p<.Ø5$) than Ø/No Swim Condition (+) or lower than the Ø/CWS Condition (*).

BL=baseline
SEM=standard error

Table 2

The effect of all doses of scopolamine and methylscopolamine pretreatment upon cold-water swim (CWS) analgesia as measured by the tail-flick test (sec).

DOSE (mg/kg)	CONDITION		POST-SWIM (min)			
			BL	30	60	120
Control						
0	No Swim (n=8)	Mean	2.30	2.39	2.42	2.34
		SEM	.121	.354	.116	.174
0	CWS (n=8)	Mean	2.37	4.63+	3.69+	2.95
		SEM	.228	.213	.209	.182
0	CWS (n=6)	Mean	2.98	5.65+	4.60+	3.93+
		SEM	.123	.191	.236	.212
Scopolamine						
0.001	CWS (n=6)	Mean	3.62	5.81+	4.75+	4.25+
		SEM	.340	.101	.258	.108
0.01	CWS (n=8)	Mean	2.36	5.10+	4.36+	3.56+
		SEM	.088	.191	.259	.177
0.05	CWS (n=6)	Mean	3.23	5.88+	4.51+	4.51+
		SEM	.322	.182	.411	.198
0.1	CWS (n=8)	Mean	2.77	5.10+	3.66+	3.14+
		SEM	.114	.232	.252	.223
0.5	CWS (n=6)	Mean	3.31	5.80+	4.85+	4.52+
		SEM	.160	.130	.265	.529
1.0	CWS (n=8)	Mean	2.39	4.83+	3.86+	3.33+
		SEM	.146	.209	.193	.124
10.0	CWS (n=8)	Mean	2.32	4.73+	3.48+	3.11+
		SEM	.104	.204	.135	.119

Table 2 (continued)

Methylscopolamine						
Ø.Ø1	CWS	Mean	3.Ø5	5.75+	4.47+	4.Ø1+
	(n=6)	SEM	.231	.139	.366	.337
Ø.1	CWS	Mean	2.38	5.72+	4.6Ø+	3.93+
	(n=6)	SEM	.248	.163	.169	.246
1.Ø	CWS	Mean	2.68	4.93+	4.12+	3.9Ø+*
	(n=8)	SEM	.161	.3Ø9	.4Ø2	.388
1Ø.Ø	CWS	Mean	2.32	5.25+	4.43+*	4.Ø1+*
	(n=8)	SEM	.212	.273	.3Ø9	.384

Significantly higher (Dunnett comparison, p<.Ø5) than Ø/No Swim Condition (+) or lower than the Ø/CWS Condition (*).

BL=baseline
SEM=standard error

Table 3

The effect of all doses of scopolamine and methylscopolamine pretreatment upon cold-water swim (CWS) hypothermia as measured by core body temperature.

DOSE (mg/kg)	CONDITION		POST-SWIM (min)			
			BL	30	60	120
Control						
0	No Swim (n=8)	Mean	36.7	36.3	36.7	36.7
		SEM	.285	.604	.284	.349
0	CWS (n=8)	Mean	37.3	29.9+	34.6	35.9
		SEM	.133	.767	.564	.409
0	CWS (n=6)	Mean	36.7	28.8+	32.4+	35.1
		SEM	.161	1.07	1.05	.448
Scopolamine						
0.001	CWS (n=6)	Mean	36.7	26.8+	31.7+	35.3+
		SEM	.280	.317	.672	.523
0.01	CWS (n=8)	Mean	37.1	27.3+*	31.2+*	33.8+*
		SEM	.228	1.38	1.37	1.11
0.05	CWS (n=6)	Mean	37.0	27.9+	32.3	34.5+
		SEM	.343	.767	.289	.204
0.1	CWS (n=8)	Mean	36.8	29.0+*	34.1+	35.6
		SEM	.239	.854	1.00	.656
0.5	CWS (n=6)	Mean	35.4	28.0+	31.9+	33.9+
		SEM	1.88	.611	.743	.642
1.0	CWS (n=8)	Mean	36.9	27.6+*	32.6+*	35.3
		SEM	.334	.575	.918	.515
10.0	CWS (n=8)	Mean	36.7	30.1+	33.4+	35.9
		SEM	.424	1.35	.930	.374

Table 3 (continued)

Methylscopolamine						
Ø.Ø1	CWS (n=6)	Mean	36.7	27.8+	31.9+	34.6+
		SEM	.249	1.11	.762	.645
Ø.1	CWS (n=6)	Mean	36.7	28.9+	33.3+	35.3
		SEM	.Ø72	.782	.535	.431
1.Ø	CWS (n=8)	Mean	37.6	3Ø.7+	34.3+	35.8
		SEM	.176	.755	.699	.783
1Ø.Ø	CWS (n=8)	Mean	37.2	28.6+	32.7+*	35.Ø
		SEM	.254	1.22	1.Ø8	.6Ø2

Significantly higher (Dunnett comparison, p<.Ø5) than Ø/No Swim Condition (+) or lower than the Ø/CWS Condition (*).

BL=baseline
SEM=standard error

Table 4

Effect of scopolamine and methylscopolamine upon forepaw footshock (FPS) analgesia as measured by the tail-flick test (sec).

DOSE (mg/kg)	CONDITION		POST-SHOCK (min) BL	Ø	1	2	4
Control							
Ø	No FPS	Mean	3.Ø8	3.24	2.87	2.72	2.78
		SEM	.711	.345	.151	.135	.2Ø7
Ø	FPS	Mean	3.Ø3	6.41+	5.Ø8+	4.Ø6	4.18
		SEM	.263	.942	.512	.479	.426
Scopolamine							
Ø.Ø1	FPS	Mean	2.99	6.53+	5.74+	5.58	5.15
		SEM	.23Ø	1.Ø3	.81Ø	1.Ø1	1.Ø4
Ø.1Ø	FPS	Mean	3.14	7.84	6.94	6.68	4.6Ø
		SEM	.267	1.Ø8	1.Ø5	1.16	.844
1.Ø	FPS	Mean	3.Ø4	8.36	5.24	4.97	3.87
		SEM	1.21	.891	.794	.827	.221
1Ø.Ø	FPS	Mean	3.19	6.82	4.96	4.33	4.25
		SEM	.263	.9Ø9	.867	.851	.264
Methylscopolamine							
1.Ø	FPS	Mean	3.Ø4	8.24	6.Ø9	4.62	4.Ø9
		SEM	.141	.866	1.Ø1	.49Ø	.688
1Ø.Ø	FPS	Mean	2.99	7.2Ø	5.31	4.69	3.78
		SEM	.341	.94Ø	1.Ø5	.821	.489

Significantly higher (Dunnett comparison, $p<.05$) than either the Ø/No Shock condition (+) or the Ø/Shock condition (*).

BL=baseline
SEM=standard error

Table 4 (continued)

DOSE (mg/kg)	CONDITION		POST-SHOCK (min)			
			6	8	10	12
Control						
0	No FPS	Mean	2.78	2.86	3.03	2.68
		SEM	.198	.172	.341	.151
0	FPS	Mean	4.02	3.51	3.90	3.85
		SEM	.432	.390	.461	.415
Scopolamine						
0.01	FPS	Mean	4.65	4.66	4.41	4.13
		SEM	.895	.832	.851	.290
0.10	FPS	Mean	4.78	5.03	4.28	3.85
		SEM	.709	.895	.687	.632
1.0	FPS	Mean	3.47	3.86	4.06	3.93
		SEM	.221	.531	.452	.894
10.0	FPS	Mean	3.82	4.10	3.80	4.13
		SEM	.493	.651	.410	.689
Methylscopolamine						
1.0	FPS	Mean	5.29	3.91	4.18	4.23
		SEM	.798	.445	.866	.741
10.0	FPS	Mean	4.60	3.84	3.51	3.50
		SEM	.855	.444	.431	.513

Table 4 (continued)

DOSE (mg/kg)	CONDITION		POST-SHOCK (min)			
			14	16	18	20
Control						
Ø	No FPS	Mean	3.68	2.87	3.73	3.11
		SEM	.939	.214	.910	.315
Ø	FPS	Mean	3.50	3.34	3.36	3.23
		SEM	.480	.377	.426	.374
Scopolamine						
Ø.Ø1	FPS	Mean	4.74	3.58	3.66	3.63
		SEM	.837	.286	.205	.346
Ø.1Ø	FPS	Mean	3.25	3.83	3.67	2.98
		SEM	.192	.563	.497	.327
1.Ø	FPS	Mean	3.54	3.95	3.12	2.87
		SEM	.335	.518	.239	.167
1Ø.Ø	FPS	Mean	3.33	3.50	3.42	3.28
		SEM	.634	.287	.590	.435
Methylscopolamine						
1.Ø	FPS	Mean	3.91	3.51	3.67	3.31
		SEM	.263	.314	.444	.341
1Ø.Ø	FPS	Mean	4.63	3.26	3.84	3.21
		SEM	.888	.477	.548	.380

Table 5

Effect of scopolamine and methylscopolamine on hindpaw footshock (HPS) analgesia as measured by the tail-flick test (sec).

DOSE (mg/kg)	CONDITION		POST-SHOCK (min)				
			BL	Ø	1	2	4
Control							
Ø	No HPS	Mean	3.23	3.25	2.92	3.12	3.24
		SEM	.329	.231	.344	.324	.185
Ø	HPS	Mean	3.33	8.99+	7.46+	7.34+	7.43+
		SEM	2.84	.529	.840	1.01	.693
Scopolamine							
Ø.Ø1	HPS	Mean	3.57	9.63+	8.18+	7.03+	6.07+
		SEM	.271	.257	.744	.879	.889
Ø.1Ø	HPS	Mean	2.95	9.47+	8.27+	7.45+	6.19+
		SEM	.282	.532	.868	1.11	.987
1.Ø	HPS	Mean	3.34	9.47+	8.93+	8.3Ø+	7.94+
		SEM	.357	.527	.826	.Ø47	1.Ø2
1Ø.Ø	HPS	Mean	3.15	9.99+	6.91+	7.81+	6.47+
		SEM	.216	.Ø14	.767	.971	1.1Ø
Methylscopolamine							
1.Ø	HPS	Mean	3.Ø1	9.32+	6.59+	5.39+	3.93
		SEM	.262	.5Ø1	1.Ø3	.8Ø1	.481
1Ø.Ø	HPS	Mean	3.89	8.74+	7.63+	6.41+	4.44
		SEM	.475	.799	1.Ø3	.935	.851

Significantly higher (Dunnett comparison, $p<.05$) than either the Ø/No Shock condition (+) or lower or higher than the Ø/Shock condition (*).

BL=baseline
SEM=standard error

Table 5 (continued)

DOSE (mg/kg)	CONDITION		POST-SHOCK (min)			
			6	8	10	12
Control						
Ø	No HPS	Mean	3.2Ø	3.16	3.37	2.89
		SEM	.26Ø	.355	.233	.234
Ø	HPS	Mean	6.22+	5.88+	6.11+	6.55
		SEM	1.Ø3	1.Ø4	.72Ø	.88Ø
Scopolamine						
Ø.Ø1	HPS	Mean	7.25+	6.73+	6.74+	7.24+
		SEM	.738	.958	.794	.763
Ø.1Ø	HPS	Mean	5.89+	5.99+	6.23+	5.62
		SEM	1.13	1.18	1.11	1.18
1.Ø	HPS	Mean	8.Ø7+	6.56+	6.Ø1+	6.79+
		SEM	.794	1.15	1.14	1.14
1Ø.Ø	HPS	Mean	6.21+	5.15	4.65	4.24
		SEM	1.11	.8Ø3	.691	.487
Methylscopolamine						
1.Ø	HPS	Mean	4.42	5.8Ø+	3.62*	6.3Ø
		SEM	.7Ø7	.916	.65Ø	1.22
1Ø.Ø	HPS	Mean	5.57+	4.54	5.74+	4.61
		SEM	.857	.664	.836	.829

Table 5 (continued)

DOSE (mg/kg)	CONDITION		POST-SHOCK (min)			
			14	16	18	20
Control						
Ø	No HPS	Mean	3.91	3.49	3.52	3.47
		SEM	.354	.250	.226	.152
Ø	HPS	Mean	6.56	5.74	6.32	6.12
		SEM	.600	.299	.924	.803
Scopolamine						
Ø.Ø1	HPS	Mean	6.57+	5.42+	4.73	5.09
		SEM	.873	.734	.399	.733
Ø.1Ø	HPS	Mean	8.Ø3+*	4.52	6.58+	5.61+
		SEM	1.Ø3	.800	1.Ø5	8.13
1.Ø	HPS	Mean	6.49+	6.47+*	5.89	6.18+
		SEM	1.2Ø	.933	.937	1.2Ø
1Ø.Ø	HPS	Mean	5.46	4.7Ø	4.24	3.93
		SEM	.596	.634	.721	.363
Methylscopolamine						
1.Ø	HPS	Mean	4.28	4.4Ø	4.Ø3	3.95
		SEM	.573	.936	.626	.549
1Ø.Ø	HPS	Mean	5.6Ø+	4.79+	4.84	4.62
		SEM	1.2Ø	1.22	1.27	1.Ø3

Table 6

Summary of the effects of scopolamine and methylscopolamine on various stressors as demonstrated by different behavioral measures.

type of analgesia	drug treatment	behavioral measure	results
Experiment 1A			
CWS	SCOP and METHYL	FJ	decrease
CWS	SCOP and METHYL	TF	increase
CWS	SCOP and METHYL	CT	increase
Experiment 1B			
2DG	SCOP and METHYL	FJ	increase
2DG	SCOP and METHYL	TF	no effect
2DG	SCOP and METHYL	FI	decrease
Experiment 1C			
FPS	SCOP and METHYL	TF	no effect
HPS	SCOP and METHYL	TF	no effect
Experiment 2			
PILO	SCOP	FJ	decrease
PILO	METHYL	FJ	no effect
PILO	SCOP	TF	decrease
PILO	METHYL	TF	no effect
Experiment 3			
BL	SCOP and METHYL	FJ	no effect
BL	SCOP and METHYL	TF	no effect
BL	SCOP and METHYL	CT	no effect
BL	SCOP and METHYL	FI	decrease
Experiment 4			
DADL	SCOP	FJ	increase
BEND	SCOP	FJ	no effect
MOR	SCOP	FJ	increase

BEND=beta-endorphin
BL=baseline
CT=core body temperature
CWS=cold-water swim
DADL=D-ala-D-leu-enkephalin
FI=food intake
FJ=flinch jump thresholds
FPS=forepaw footshock
HPS=hindpaw footshock
METHYL=methylscopolamine
MOR=morphine
PILO=pilocarpine
SCOP=scopolamine
TF=tail-flick test
2DG=2-deoxy-D-glucose

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