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Effects of Muscarinic Receptor Antagonism Upon Two Forms of Stress-Induced Analgesia

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SPERBER, E. S., E. KRAMER AND R. J. BODNAR. *Effects of muscarinic receptor antagonism upon two forms of stress-induced analgesia.* PHARMACOL BIOCHEM BEHAV 25(1) 171–179, 1986.—The present study assessed in rats the effects of muscarinic receptor antagonism upon analgesia induced by cold-water swims (CWS: 2°C for 3.5 min) and 2-deoxy-D-glucose (2DG: 600 mg/kg). First, CWS analgesia was significantly reduced 30 min after the swim by scopolamine (0.01 and 0.1 mg/kg) and methylscopolamine (10 mg/kg) pretreatment, and was eliminated 60 min after the swim by scopolamine (0.01–10 mg/kg) and methylscopolamine (1,10 mg/kg) pretreatment. In contrast, scopolamine potentiated CWS hypothermia. Second, while scopolamine (1 mg/kg) and methylscopolamine (1,10 mg/kg) pretreatment prolonged 2DG analgesia, both antagonists dose-dependently reduced 2DG hyperphagia. Third, the changes in analgesic and hypothermic stress responses were not due to baseline shifts in jump thresholds or body temperatures. However the dose-dependent reductions by scopolamine and methylscopolamine in baseline food intake and 2DG hyperphagia were significantly correlated. Fourth, the dose-dependent reduction by scopolamine and methylscopolamine of pilocarpine analgesia differed in pattern from the other analgesic effects, suggesting heterogeneity in muscarinic receptor modulation of different analgesic responses.

Pain Analgesia Acetylcholine Scopolamine Methylscopolamine Cold-water swims
2- Deoxy-D-glucose Hypothermia Hyperphagia Rats

INCREASES in pain thresholds of rats occur following acute exposure to certain environmental stimuli, including inescapable foot shock, cold-water swims (CWS) and 2-deoxy-D-glucose (2DG) glucoprivation. These analgesic responses differ in their pharmacological, physiological and hormonal profiles (see reviews: [5, 43, 50, 56]. In assessing neurochemical substrates of these analgesic responses, several laboratories have examined the role of the muscarinic receptor in the mediation of stress-induced analgesia. Lewis and co-workers [35] found that scopolamine blocked analgesia induced by prolonged, intermittent foot shock (PIFS) delivered to all four paws, but failed to block analgesia induced by brief, continuous foot shock (BCFS) delivered to all four paws. Methylscopolamine, which fails to cross the blood-brain barrier [26] failed to alter either PIFS or BCFS analgesia. Since analgesia induced by PIFS, but not BCFS is significantly reduced by naloxone [34] and develops cross-tolerance with morphine analgesia [37], these researchers concluded that the muscarinic receptor was involved in opioid-sensitive, but not opioid-insensitive forms of pain control. MacLennan and co-workers [40] reported similar results using a different shock analgesia paradigm. Analgesia occurs in rats that are briefly re-exposed to inescapable tail shocks 24 hr after an initial prolonged exposure to inescapable tail shocks. Scopolamine significantly reduced this form of analgesia. This form of shock analgesia is also decreased by opiate receptor antagonists and is cross-tolerant with morphine analgesia [22, 30, 41]. However, Watkins and co-workers [55] reported a different pattern of results: scopolamine reduced analgesia induced by hind paw shock (HPS), but failed to affect analgesia induced by forepaw shock (FPS). FPS, but not HPS analgesia, is reduced by either peripheral or central pretreatment with naloxone [52,56]. The cholinergic mediation of HPS analgesia was presumed to be (a) centrally-mediated since methylscopolamine failed to alter this response, and (b) suprasinal since intrathecal administration of scopolamine produced minimal effects. However, these authors [55] also found that the opioid-sensitive [53,56] analgesia induced by classical conditioning was reduced by scopolamine, but not methylscopolamine, pretreatment. Thus, the sensitivity or insensitivity of different forms of shock analgesia to opioid manipulations does not always indicate whether a particular form of shock analgesia is reduced by muscarinic receptor antagonism. Rather, the effect of muscarinic receptor antagonism is apparently determined by the parametric variables of shock. These parametric variables also appear to determine whether the subsequent analgesia is dependent upon hormonal control: hypophysectomy reduces both PIFS and shock re-exposure analgesia, but fails to affect BCFS, FPS or HPS analgesia [34, 36, 39, 54, 56].

The duration of the analgesic effect is another critical parametric variable to be considered in the classification of analgesic responses induced by selective environmental

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TABLE 1

ALTERATIONS IN COLD-WATER SWIM (CWS: 2°C FOR 3.5 MIN) ANALGESIA ON THE JUMP TEST (mA) FOLLOWING SCOPOLAMINE OR METHYLSCOPOLAMINE PRETREATMENT

Dose (mg/kg)	Condition		BL	Post-Swim (min) 30	60	120
0	No Swim	Mean	0.306	0.281	0.305	0.312
		SEM	0.011	0.014	0.014	0.016
0	CWS	Mean	0.291	0.463†	0.399†	0.390
		SEM	0.028	0.042	0.037	0.036
Scopolamine						
0.01	CWS	Mean	0.304	0.353*	0.365	0.313
		SEM	0.022	0.064	0.035	0.036
0.1	CWS	Mean	0.297	0.274*	0.301*	0.308
		SEM	0.015	0.023	0.019	0.012
1.0	CWS	Mean	0.309	0.382†	0.325	0.308
		SEM	0.008	0.027	0.026	0.024
10.0	CWS	Mean	0.306	0.384†	0.306	0.335
		SEM	0.020	0.038	0.025	0.025
Methylscopolamine						
1.0	CWS	Mean	0.304	0.328*	0.333	0.346
		SEM	0.023	0.036	0.036	0.030
10.0	CWS	Mean	0.307	0.423†	0.345	0.331
		SEM	0.012	0.060	0.020	0.023

Significant differences denoted are relative to either the corresponding 0/No Swim condition (†Dunnett comparisons, $p<0.05$) or the corresponding 0/CWS condition (*Dunn comparisons, $p<0.05$).

TABLE 2

ALTERATIONS IN CWS HYPOTHERMIA (°C) FOLLOWING SCOPOLAMINE AND METHYLSCOPOLAMINE PRETREATMENT

Dose (mg/kg)	Condition		BL	Post-Swim (min) 30	60	120
0	No Swim	Mean	36.7	36.3	36.7	36.7
		SEM	0.3	0.6	0.3	0.3
0	CWS	Mean	37.3	29.9†	34.6†	35.9
		SEM	0.1	0.8	0.6	0.4
Scopolamine						
0.01	CWS	Mean	37.1	27.3*†	31.2*†	33.8*†
		SEM	0.2	1.4	1.4	1.1
0.1	CWS	Mean	36.8	29.0†	34.1†	35.6
		SEM	0.2	0.9	1.0	0.7
1.0	CWS	Mean	36.9	27.6*†	32.6*†	35.3
		SEM	0.3	0.6	0.9	0.5
10.0	CWS	Mean	36.7	30.1†	33.4†	35.9
		SEM	0.4	1.4	0.9	0.4
Methylscopolamine						
1.0	CWS	Mean	37.6	30.7†	34.3†	35.8
		SEM	0.2	0.8	0.7	0.8
10.0	CWS	Mean	37.2	28.6†	32.7*†	35.0†
		SEM	0.3	1.2	1.1	0.6

Significant differences denoted are relative to either the corresponding 0/No Swim condition (†Dunnett comparisons, $p<0.05$) or the corresponding 0/CWS condition (*Dunn comparisons, $p<0.05$).

stimuli. The duration of the different forms of shock analgesia is typically 20–30 min. Two other stressors, CWS and 2DG, elicit analgesic responses in rats that last for up to 2 hr [7,8]. Both stressors exhibit different pharmacological and physiological analgesic profiles from each other and also from shock analgesia. Although CWS and 2DG analgesia develop full and reciprocal cross-tolerance [49], only 2DG analgesia develops cross-tolerance with morphine analgesia [13,49]. Further, neither CWS nor 2DG analgesia is significantly reduced by naloxone [9,11]. However, both CWS and 2DG analgesia are altered by hormonal manipulations, although in opposite directions. Reductions in CWS analgesia [1–3] and increases in 2DG analgesia [1,10] occur following destruction of either the medial-basal hypothalamus or the pituitary gland.

The present study assessed in rats the effects of scopolamine and methylscopolamine pretreatment upon CWS and 2DG analgesia. Previous preliminary studies found that physostigmine and CWS analgesia in mice are reduced by benactyzine, a general cholinergic antagonist [32,46]. Further, mice acutely exposed to CWS display elevated levels of acetylcholine in cerebellum, striatum and cortex, and elevated levels of choline in cerebellum, striatum, cortex and hippocampus [47]. The first experiment evaluated the effects of scopolamine and methylscopolamine upon CWS analgesia on the jump test [23]. CWS hypothermia was also examined to evaluate whether muscarinic receptor antagonism produced similar alterations in a second stress response following CWS. Previous studies indicate that other physiological and pharmacological manipulations produced either similar [1,17] or dissimilar [3, 12, 15, 16] effects upon CWS analgesia and CWS hypothermia. The second experiment evaluated the effects of scopolamine and methylscopolamine upon 2DG analgesia. 2DG hyperphagia was also examined to evaluate whether muscarinic receptor antagonism produced similar alterations in a second response following 2DG. Dissociations between 2DG analgesia and 2DG hyperphagia have been observed following other physiological and pharmacological manipulations (e.g., [4,14]). If scopolamine or methylscopolamine produced changes in any response following CWS or 2DG, the changes could be due to either specific interactions with that stress measure, or alternatively reflect a shift in baseline thresholds by scopolamine or methylscopolamine alone. To control for the latter possibility, the third experiment evaluated whether scopolamine or methylscopolamine alone altered jump thresholds, core body temperatures or food intake. The fourth experiment evaluated whether analgesia induced by the muscarinic receptor agonist, pilocarpine [27,28], was altered by scopolamine or methylscopolamine. This provided a standard of muscarinic antagonist action with which to compare any effects observed upon CWS and 2DG analgesia.

EXPERIMENT 1

METHOD

Subjects

Sixty-four male albino Sprague-Dawley rats (300–450 g, Queens College Breeding Colony) were housed in pairs in wire mesh cages (35×20×18 cm) and were maintained on a

12 hr light:12 hr dark cycle. Purina rat chow and water were available ad lib. The ambient temperature of the housing and experimental rooms in this and all subsequent experiments varied between 22° and 25°C. All testing in this and all subsequent experiments occurred between 2 and 6 hr into the light cycle.

Initial Procedure

Jump thresholds [23] were determined according to an ascending method of limits procedure in which electric shocks were delivered by a 60 Hz constant current shock generator and a grid scrambler through a 30 cm by 24 cm floor composed of 16 grids. Each trial began with the animal receiving a 300 msec foot shock at a current intensity of 0.1 mA. Subsequent shocks occurred at 10 sec intervals and were increased in equal 0.05 mA steps until the jump threshold was determined. The jump threshold was defined in mA as the lowest of two consecutive intensities in which the animal simultaneously removed both hindpaws from the grids. After each trial, the current intensity was reset to 0.1 mA for the next trial until 6 trials were completed.

Protocol

Eight groups of eight rats each were matched on the basis of four days of baseline jump thresholds. The first four groups received intraperitoneal injections of scopolamine hydrobromide (Sigma Company) at doses of either 0.01, 0.1, 1.0 or 10.0 mg/kg respectively 5 min prior to a 3.5 min swim in a 2°C temperature bath. The fifth and sixth groups received intraperitoneal injections of scopolamine methyl nitrate (methylscopolamine; Sigma Company) at doses of either 1 or 10 mg/kg respectively 5 min prior to the swim condition. The seventh group received a vehicle injection (1 ml normal saline/kg body weight, IP) 5 min prior to the swim condition. The eighth group received neither the injection nor the swim. Jump thresholds and core body temperatures were determined 30, 60 and 120 min following the swim. Core body temperatures were determined by inserting the rectal probe (5 cm) of a digital thermometer (Bailey Instruments: sensitivity—0.1°C) until a stable reading was achieved.

RESULTS

CWS Analgesia

Significant differences in jump thresholds were observed across test times, $F(3,168)=8.24$, $p<0.0001$, and for the interaction between groups and test times, $F(21,168)=1.88$, $p<0.016$, but not among groups, $F(7,56)=1.69$. Table 1 indicates that CWS significantly increased jump thresholds over no-swim values at 30 and 60 min in vehicle-pretreated rats, and at 30 min in rats pretreated with either the 1 and 10 mg/kg doses of scopolamine or the 10 mg/kg dose of methylscopolamine (Dunnett comparisons, $p<0.05$). In contrast, CWS failed to increase jump thresholds over no-swim values in rats pretreated with either the 0.01 and 0.1 mg/kg doses of scopolamine or the 1 mg/kg dose of methylscopolamine (Dunnett comparisons, $p<0.05$). Moreover, the magnitude of CWS analgesia was significantly reduced in rats receiving either the 0.01 (30 min) and 0.1 (30 and 60 min) mg/kg doses of scopolamine or the 1 mg/kg (30 min) dose of methylscopolamine (Dunn comparisons, $p<0.05$). Thus, while some doses of scopolamine and methylscopolamine significantly reduced CWS analgesia at its peak effect (30 min), clear dose-dependent effects were not observed. In contrast, all scopolamine and methylscopolamine doses eliminated the residual analgesia observed 60 min following CWS in vehicle-pretreated rats, suggesting that muscarinic receptor antagonism is more effective in reducing the duration, rather than the peak magnitude of CWS analgesia on the jump test.

TABLE 3

ALTERATIONS IN 2-DEOXY-D-GLUCOSE (2DG: 600 mg/kg) ANALGESIA ON THE JUMP TEST (mA) FOLLOWING SCOPOLAMINE AND METHYLSCOPOLAMINE PRETREATMENT

Dose (mg/kg)	Condition		Post-Injection (min) BL	30	60	120
0	Vehicle	Mean	0.287	0.294	0.320	0.289
		SEM	0.020	0.013	0.009	0.014
0	2DG	Mean	0.290	0.438†	0.437†	0.354
		SEM	0.021	0.036	0.042	0.036
Scopolamine						
0.01	2DG	Mean	0.281	0.415†	0.424†	0.374
		SEM	0.021	0.060	0.029	0.033
0.1	2DG	Mean	0.307	0.447†	0.408†	0.365
		SEM	0.011	0.038	0.033	0.028
1.0	2DG	Mean	0.305	0.533†	0.444†	0.397†
		SEM	0.020	0.049	0.056	0.032
10.0	2DG	Mean	0.307	0.409†	0.431†	0.373
		SEM	0.014	0.026	0.046	0.030
Methylscopolamine						
1.0	2DG	Mean	0.294	0.466†	0.444†	0.444†
		SEM	0.022	0.068	0.033	0.035
10.0	2DG	Mean	0.302	0.484†	0.457†	0.494*†
		SEM	0.014	0.045	0.041	0.042

Significant differences denoted are relative to either the corresponding 0/Vehicle condition (†Dunnett comparisons, $p<0.05$) or the corresponding 0/2DG condition (*Dunn comparisons, $p<0.05$).

CWS Hypothermia

Significant differences in core body temperatures were observed among groups, $F(7,56)=4.84$, $p<0.0001$, across test times, $F(3,168)=236.77$, $p<0.0001$, and for the interaction between groups and test times, $F(21,168)=5.44$, $p<0.0001$. Table 2 indicates that CWS significantly decreased core body temperatures below no-swim values at 30 and 60 min in rats pretreated with either vehicle, the 0.1, 1 and 10 mg/kg doses of scopolamine or the 1 mg/kg dose of methylscopolamine (Dunnett comparisons, $p<0.05$). CWS significantly decreased core body temperatures below no-swim values across the time course in rats pretreated with either the 0.01 mg/kg dose of scopolamine or the 10 mg/kg dose of methylscopolamine (Dunnett comparisons, $p<0.05$). Moreover, the magnitude of CWS hypothermia was significantly potentiated in rats receiving either the 0.01 (30, 60 and 120 min) and the 1.0 (30 and 60 min) mg/kg doses of scopolamine or the 10 mg/kg (60 min) dose of methylscopolamine. Thus, while scopolamine and methylscopolamine increased both the duration and magnitude of CWS hypothermia, these effects did not exhibit clear dose-response patterns.

TABLE 4

DOSE-DEPENDENT REDUCTION OF 2DG HYPERPHAGIA (g) FOLLOWING SCOPOLAMINE AND METHYLSCOPOLAMINE PRETREATMENT

Dose (mg/kg)		Intake (g, SEM)
0	Vehicle	4.00 (0.62)
0	2DG	9.97† (0.66)
Scopolamine		
0.01	2DG	10.11† (1.16)
0.1	2DG	8.99† (0.59)
1.0	2DG	6.14* (0.86)
10.0	2DG	4.17* (0.67)
Methylscopolamine		
1.0	2DG	5.50* (0.96)
10.0	2DG	3.02* (0.68)

Significant differences denoted are relative to either the corresponding 0/Vehicle condition (†Dunnett comparisons, $p<0.05$) or the corresponding 0/2DG condition (*Dunn comparisons, $p<0.05$).

TABLE 5

ALTERATIONS IN JUMP THRESHOLDS (mA) FOLLOWING SCOPOLAMINE OR METHYLSCOPOLAMINE PRETREATMENT

Dose (mg/kg)		Post-Injection (min)		
	BL	30	60	120
0	Mean 0.281	0.281	0.264	0.294
	SEM 0.026	0.020	0.016	0.018
Scopolamine				
0.01	Mean 0.334	0.315	0.343†	0.306
	SEM 0.033	0.029	0.032	0.032
0.1	Mean 0.268	0.267	0.343†	0.332
	SEM 0.017	0.022	0.027	0.027
1.0	Mean 0.295	0.226	0.242	0.264
	SEM 0.021	0.029	0.042	0.031
10.0	Mean 0.278	0.233	0.221	0.263
	SEM 0.024	0.025	0.028	0.027
Methylscopolamine				
1.0	Mean 0.285	0.329	0.322	0.333
	SEM 0.015	0.020	0.025	0.028
10.0	Mean 0.274	0.325	0.301	0.303
	SEM 0.014	0.027	0.028	0.034

Significant differences denoted are relative to the corresponding Vehicle condition (†Dunnett comparisons, $p<0.05$).

EXPERIMENT 2

METHOD

Protocol

Sixty-four naive male rats were assigned to eight groups of eight rats each on the basis of their baseline jump thresholds as described previously. The first seven groups received scopolamine (0.01, 0.1, 1.0, 10.0 mg/kg, IP), methylscopolamine (1, 10 mg/kg, IP) or vehicle respectively 5 min prior to an injection of a 600 mg/kg dose of 2DG (Sigma Company; 600 mg 2DG/2 ml normal saline/kg body weight, IP). The eighth group received two vehicle injections spaced 5 min apart. Jump thresholds were determined 30, 60 and 120 min following the 2DG injection. All animals were then housed individually in wire mesh cages under the same ad lib conditions. One week after jump threshold testing, the eight groups received the same experimental treatments, and were then given preweighed pellets of rat chow (Purina) and water. Food intake was calculated 5 hr later to the nearest 0.1 g (Sartorious Balance) with spillage accounted for by measuring the food residues collected beneath the wire mesh cage.

RESULTS

2DG Analgesia

Significant differences in jump thresholds were observed among groups, $F(7,56)=2.40$, $p<0.032$, across test times, $F(3,168)=46.54$, $p<0.0001$, and for the interaction between groups and test times, $F(21,168)=1.82$, $p<0.021$. Table 3 indicates that 2DG significantly increased jump thresholds over control values at 30 and 60 min in rats pretreated with either vehicle or the 0.01, 0.1 and 10 mg/kg doses of scopolamine (Dunnett comparisons, $p<0.05$). 2DG significantly increased jump thresholds over control values across the time course in rats pretreated with either the 1 mg/kg dose of scopolamine or the 1 and 10 mg/kg doses of methylscopolamine (Dunnett comparisons, $p<0.05$). Moreover, the magnitude of 2DG analgesia was significantly potentiated at 120 min in rats receiving the 10 mg/kg dose of methylscopolamine (Dunn comparison, $p<0.05$). Thus, in contrast to the reductions in CWS analgesia by scopolamine and methylscopolamine, it appears that the duration of 2DG analgesia is increased by muscarinic receptor antagonists.

2DG Hyperphagia

Table 4 summarizes the significant differences in food intake among groups, $F(7,56)=12.61$, $p<0.0001$, in which both scopolamine and methylscopolamine produced dose-dependent decreases in 2DG hyperphagia. 2DG significantly increased food intake over control values in rats pretreated with either vehicle or the 0.01 and 0.1 mg/kg doses of scopolamine (Dunnett comparisons, $p<0.05$). 2DG failed to alter food intake from control values in rats pretreated with the 1 and 10 mg/kg doses of either scopolamine or methylscopolamine (Dunnett comparisons, $p<0.05$). The magnitude of 2DG hyperphagia was significantly reduced in rats pretreated with the 1 and 10 mg/kg doses of either scopolamine or methylscopolamine (Dunn comparisons, $p<0.05$).

EXPERIMENT 3

METHOD

Protocol

Forty-two naive male rats were assigned to seven groups of six rats each on the basis of their baseline jump thresholds as described previously. The seven groups received

TABLE 6

DOSE-DEPENDENT REDUCTION OF FOOD INTAKE (g) FOLLOWING SCOPOLAMINE AND METHYLSCOPOLAMINE PRETREATMENT

Dose (mg/kg)	Intake (g, SEM)
0	3.93 (0.90)
Scopolamine	
0.01	3.02 (0.77)
0.1	2.37 (0.43)
1.0	0.98† (0.29)
10.0	1.08† (0.21)
Methylscopolamine	
1.0	0.93† (0.38)
10.0	0.98† (0.20)

Significant differences denoted are relative to the corresponding Vehicle condition (†Dunnett comparisons, $p<0.05$).

TABLE 7

ALTERATIONS IN PILOCARPINE (PILO: 10 mg/kg, IP) ANALGESIA ON THE JUMP TEST (mA) FOLLOWING SCOPOLAMINE OR METHYLSCOPOLAMINE PRETREATMENT

Dose (mg/kg)	Condition		Post-Injection (min) BL	30	60	120
0	Vehicle	Mean	0.339	0.346	0.325	0.345
		SEM	0.016	0.014	0.022	0.015
0	PILO	Mean	0.339	0.468†	0.432†	0.374
		SEM	0.016	0.026	0.018	0.013
Scopolamine						
0.01	PILO	Mean	0.281	0.491†	0.333	0.311
		SEM	0.020	0.115	0.039	0.026
0.1	PILO	Mean	0.271	0.446	0.407	0.357
		SEM	0.022	0.028	0.032	0.040
1.0	PILO	Mean	0.285	0.338*	0.318*	0.327
		SEM	0.027	0.042	0.045	0.034
10.0	PILO	Mean	0.311	0.250*	0.310*	0.353
		SEM	0.024	0.019	0.025	0.029
Methylscopolamine						
1.0	PILO	Mean	0.307	0.471†	0.474†	0.469
		SEM	0.020	0.057	0.051	0.053
10.0	PILO	Mean	0.313	0.426	0.421	0.399
		SEM	0.027	0.057	0.046	0.044

Significant differences denoted are relative to either the corresponding 0/Vehicle condition (†Dunnett comparisons, $p<0.05$) or the corresponding 0/PILO condition (*Dunn comparisons, $p<0.05$).

scopolamine (0.01, 0.1, 1.0, 10.0 mg/kg, IP), methylscopolamine (1, 10 mg/kg, IP) or vehicle respectively with jump thresholds and core body temperatures determined 30, 60 and 120 min following the injection. One week later, the seven groups received the same experimental treatments, and underwent the same feeding paradigm described in Experiment 2, except that the 2DG injection was not administered. Food intake was calculated 5 hr later as described previously.

RESULTS

Jump Thresholds

Significant differences in jump thresholds were observed for the interaction between groups and test times, $F(18,105)=2.53$, $p<0.0017$, but not among groups, $F(6,35)=1.77$, or across test times, $F(3,105)=1.24$. Table 5 indicates that jump thresholds were significantly increased over vehicle values at 60 min following only the 0.01 and 0.1 mg/kg doses of scopolamine (Dunnett comparisons, $p<0.05$). Thus, it appears that the transient increase in jump thresholds induced by scopolamine alone does not correspond with either the observed reductions in CWS analgesia or the observed potentiations in 2DG analgesia induced by scopolamine and methylscopolamine pretreatment.

Core Body Temperatures

Significant differences in core body temperatures were observed across test times, $F(3,105)=4.17$, $p<0.008$, but not among groups, $F(6,35)=1.27$, or for the interaction between groups and test times, $F(18,105)=1.17$. Although a small but significant decrease (0.4°C) in core body temperatures occurred in all groups across the time course, the individual treatment groups failed to differ from each other (Dunnett comparisons, $p<0.05$). Thus, it appears that the lack of scopolamine and methylscopolamine effects upon core body temperature does not correspond with the observed potentiation in CWS hypothermia.

Food Intake

Table 6 summarizes the significant differences in food intake among groups, $F(6,35)=5.47$, $p<0.0004$, in which scopolamine and methylscopolamine produced dose-dependent decreases in baseline food intake. Food intake failed to differ from control values in rats pretreated with the 0.01 and 0.1 mg/kg doses of scopolamine (Dunnett comparisons, $p<0.05$). Food intake was significantly decreased below control values in rats pretreated with the 1 and 10 mg/kg doses of either scopolamine or methylscopolamine (Dunnett comparisons, $p<0.05$). The decreases in baseline food intake and 2DG hyperphagia by scopolamine and methylscopolamine were significantly correlated with each other, $r(5)=0.893$, $p<0.02$. This effect accounted for 80% of the variance, and suggested that the effects of muscarinic receptor antagonism on the two procedures are related to each other.

EXPERIMENT 4

METHOD

Protocol

Forty-eight naive male rats were assigned to eight groups of six rats each on the basis of their baseline jump thresholds as described previously. The first seven groups received scopolamine (0.01, 0.1, 1.0, 10.0 mg/kg, IP), methylscopolamine (1, 10 mg/kg, IP) or vehicle respectively 5 min prior to an injection of a 10 mg/kg dose of pilocarpine hydrobromide (Sigma Company: 10 mg pilocarpine/ml normal saline/kg body weight, IP). The eighth group received two vehicle injections spaced 5 min apart. Jump thresholds

were determined 30, 60 and 120 min following the last injection.

RESULTS

Jump Thresholds

Significant differences in jump thresholds were observed among groups, $F(7,40)=2.26$, $p<0.049$, across test times, $F(3,120)=15.87$, $p<0.0001$, and for the interaction between groups and test times, $F(21,120)=2.56$, $p<0.0001$. Table 7 indicates that pilocarpine significantly increased jump thresholds over control values in rats pretreated with either vehicle (30 and 60 min), the 0.01 mg/kg dose of scopolamine (30 min), or the 1 mg/kg dose of methylscopolamine (30 and 60 min) (Dunnett comparisons, $p<0.05$). Pilocarpine failed to alter jump thresholds above control values in rats pretreated with either the 0.1, 1 and 10 mg/kg doses of scopolamine or the 10 mg/kg dose of methylscopolamine (Dunnett comparisons, $p<0.05$). The magnitude of pilocarpine was significantly reduced at 30 and 60 min in rats pretreated with the 1 and 10 mg/kg doses of scopolamine. Although both muscarinic receptor antagonists dose-dependently reduced pilocarpine analgesia, lower scopolamine doses were more effective in reducing (0.01 mg/kg) or eliminating (0.1 mg/kg) pilocarpine analgesia than the elimination of the effect by the 10 mg/kg dose, but not the 1 mg/kg dose, of methylscopolamine.

GENERAL DISCUSSION

Pretreatment with the muscarinic receptor antagonists, scopolamine and methylscopolamine, produced differential effects upon CWS and 2DG analgesia. The reductions in CWS analgesia by both antagonists are similar to previous reports of reductions of this effect in mice by the general cholinergic antaonist, benactyzine [32,46]. These results stand in contrast to the potentiation of the duration of 2DG analgesia by scopolamine and methylscopolamine. These changes in analgesic responses were not accompanied by similar alterations in other stress responses. Indeed, scopolamine and methylscopolamine potentiated CWS hypothermia, and both antagonists dose-dependently reduced 2DG hyperphagia. The above effects will be considered in terms of: (a) effects induced by scopolamine and methylscopolamine alone; (b) dissociations in different stress responses; (c) differentiations between CWS and 2DG analgesia and the lack of distinct dose-response patterns; and (d) an overall evaluation of cholinergic mediation of different forms of stress-induced analgesia.

A. CONTROL STUDIES

The observation that lower doses of scopolamine produced transient increases in jump thresholds agree with previous observations on the hot-plate test [51], but differ from hyperalgesic effects on the tail-flick and spatial preference tests [27, 29, 55]. These data argue against the possibility that the changes in CWS and 2DG analgesia by scopolamine and methylscopolamine were due to shifts in baseline jump thresholds by the antagonists. The analgesic effect of scopolamine alone 60 min after injection stands in contrast to the ability of both muscarinic receptor antagonists to eliminate CWS analgesia 60 min after the swim and potentiate 2DG analgesia 120 min after the 2DG injection. The failure to observe systematic changes in core body temperature following scopolamine and methylscopolamine is in contrast to a previous observation of hyperthermia (0.7°C) 1 hr following a 10 mg/kg dose of scopolamine [31]. However, strain differences in the present (Sprague-Dawley) and previous (Wistar) studies might explain this discrepency. Further, both scopolamine and methylscopolamine are capable of eliciting either hyperthermia (1.4°C) or hypothermia (1.8°C) depending upon the species, route of administration and dose range (see review: [21]). In any case, the lack of thermoregulatory effects of both muscarinic receptor antagonists cannot account for their influence in increasing the magnitude and duration of CWS hypothermia. In contrast, muscarinic receptor antagonism produces dose-dependent decreases in both baseline food intake and 2DG hyperphagia. These effects are significantly correlated and account for 80% of the variance.

B. DISSOCIATIONS OF STRESS RESPONSES

Scopolamine and methylscopolamine produced significant reductions in CWS analgesia, yet potentiated CWS hypothermia. While these effects could be observed 30 min after the swim at some antagonist doses, it was more apparent at longer post-swim intervals. While all scopolamine and methylscopolamine doses eliminated CWS analgesia 60 min after the swim, CWS hypothermia was potentiated by scopolamine (0.01 mg/kg: 60 and 120 min; 1 mg/kg: 60 min) and methylscopolamine (10 mg/kg: 60 and 120 min). These results argue against the possibility that CWS analgesia is merely an epiphenomenon of CWS hypothermia as suggested by the observation that decreases in bath temperature increase the magnitude of CWS analgesia [3]. Whatever relationship may exist between CWS analgesia and CWS hypothermia appears to be complex. Both responses are potentiated in clonidine-pretreated rats [17], and are reduced in rats with medial-basal hypothalamic damage [1]. Further, reductions in CWS analgesia, but not CWS hypothermia, are observed following repeated exposure to CWS [12], following hypophysectomy [3], and following D-phenylalanine pretreatment [15]. In contrast, increased in CWS analgesia, but not CWS hypothermia occur in rats pretreated with desipramine [16]. While the two responses are dissociated by muscarinic receptor antagonism, the mechanisms of action are not clear.

Dissociations between glucoprivic responses were also observed following scopolamine and methylscopolamine; 2DG analgesia was potentiated and 2DG hyperphagia was reduced by muscarinic receptor antagonism. These data agree with many other instances in which these two responses have been differentially affected by other pharmacological and physiological manipulations (e.g., [4,14]). While it is difficult to discern how muscarinic receptor antagonism alters 2DG analgesia, the related decreases in baseline food intake and 2DG hyperphagia by scopolamine and methylscopolamine suggest a common inhibition of peripheral parasympathetic nervous system activity. The observed dissociated effects upon the two CWS responses and the two 2DG responses suggest that the changes induced by scopolamine and methylscopolamine upon the analgesic responses of each stressor were due to changes in pain-inhibitory circuits rather than overall alterations in the perception of the stressful stimulus.

C. DIFFERENTIATIONS BETWEEN CWS AND 2DG ANALGESIA

Scopolamine and methylscopolamine pretreatment reduced CWS analgesia and potentiated 2DG analgesia despite

the comparable magnitude of each effect in vehicle-treated rats (see also, [7,8]). However, these differential effects upon CWS and 2DG analgesia were not consistently dose-dependent. This raises questions as to whether such effects are reliable, and the mechanism of receptor action through which the effects are mediated. Standard dose-response relationships were observed in the antagonism of pilocarpine analgesia by scopolamine and methylscopolamine. Further, the predominant central mechanism of action of pilocarpine analgesia [27–29] was suggested by the greater effectiveness of lower scopolamine doses to reduce or eliminate the analgesic effect as compared to methylscopolamine. For CWS analgesia, all doses of scopolamine and methylscopolamine eliminated this effect 60 min after the swim. The magnitude of CWS analgesia at this interval was 31% above no-swim values in vehicle-treated rats. Following scopolamine and methylscopolamine pretreatment, CWS failed to elevate thresholds over 10% above no-swim values, except for rats pretreated with either the 0.01 mg/kg dose of scopolamine (19%) or the 10 mg/kg dose of methylscopolamine (13%). At 30 min following the swim, CWS analgesia was significantly reduced in rats pretreated with either the 0.01 mg/kg (26%) or 0.1 mg/kg (eliminated) doses of scopolamine and the 1 mg/kg (17%) dose of methylscopolamine. Although other dose groups pretreated with scopolamine or methylscopolamine displayed significant CWS analgesia which failed to differ from CWS analgesia observed following vehicle pretreatment, there appeared to be some decrease in the magnitude of the effect: vehicle (65%), scopolamine (1 mg/kg: 36%; 10 mg/kg: 37%) and methylscopolamine (10 mg/kg: 51%). Even if these latter effects achieved statistical significance by increasing the sample size of each group, it would still leave a disparity in individual dose effectiveness. The strongest statement that can be made from the present results is that muscarinic receptor antagonism appears to be more effective in reducing the duration rather than the peak magnitude of CWS analgesia.

A similar clear lack of dose-response consistency was observed in the potentiation of 2DG analgesia by scopolamine and methylscopolamine. Scopolamine and methylscopolamine failed to alter 2DG analgesia 30 and 60 min after injection. Further, 2DG-induced changes in jump thresholds above control values at 120 min after injection were similar in rats pretreated with either vehicle (22%) or scopolamine doses of 0.01 (29%), 0.1 (26%) and 10 (29%) mg/kg. However, potentiated analgesia was observed at this interval in rats pretreated with either scopolamine (1 mg/kg: 37%) or methylscopolamine (1 mg/kg: 54%; 10 mg/kg: 71%). A more definitive analysis of the potentiation and the dose-response characteristics of this effect are hampered by the 120 min endpoint of the time course which was chosen because of previously-determined dissipation of 2DG analgesia [7].

Both scopolamine and methylscopolamine were active in altering CWS and 2DG analgesia. Scopolamine is capable of interacting with both central and peripheral muscarinic receptors. Methylscopolamine is far less effective in crossing the blood-brain barrier [26], and presumably has a peripheral site of action. Despite the lack of consistent dose-response patterns for each antagonist, the reduction of CWS analgesia and the potentiation of 2DG analgesia by both muscarinic receptor antagonists may suggest peripheral mediation of the effects. There is evidence that both CWS and 2DG analgesia are mediated in part by peripheral hormonal mechanisms, and that manipulations of these mechanisms produce dissociations in the analgesic effects similar to what is observed following muscarinic receptor antagonism. Destruction of either the medial-basal hypothalamus or the pituitary gland reduces CWS analgesia and potentiates 2DG analgesia [1, 3, 10]. The cholinergic system interacts with pituitary function. Cholinesterase inhibitors increase corticosterone [33] and adrenocorticotrophic hormone [20], as well as alter the hypothalamic-pituitary axis [20,48]. Further, the most consistent effects of muscarinic receptor antagonists occurred 60 min following CWS and 120 min following 2DG. If these effects were due to peripheral changes in hormonal function, this would suggest that the hormonal component is responsible for the maintainance and not the initiation of each analgesic response. Further pharmacokinetic studies are necessary to determine the locus of muscarinic antagonist action upon CWS and 2DG analgesia.

D. EVALUATION OF MUSCARINIC RECEPTOR INFLUENCES IN STRESS-INDUCED ANALGESIA

The present data provide further evidence for a heterogeneous role for the muscarinic receptor in stress-induced analgesia. Scopolamine, but not methylscopolamine dose-dependently reduced PIFS and HPS analgesia as well as analgesia induced by classical conditioning and shock re-exposure [35, 40, 55]. Both scopolamine and methylscopolamine reduce CWS analgesia and prolong 2DG analgesia. Yet neither antagonist affects BCFS or FPS analgesia [35,55]. Consideration of the above effects do not allow simply-postulated sites of action. The contention [35] that opioid-sensitive stressors only display decreases in analgesia following muscarinic receptor antagonism is not supported by the lack of effect upon opioid-sensitive FPS analgesia, the potentiation of opioid-sensitive 2DG analgesia, or the reduction of opioid-insensitive CWS and HPS analgesia (present results, [55]). The possible dichotomy of neural and neurohormonal mediation of stress-induced analgesia is also not totally predictive of muscarinic receptor interactions. Analgesia induced by CWS, PIFS and shock re-exposure are all dependent upon hypophysial factors and common adrenocortical modulation [3, 19, 24, 25, 34, 36, 38, 39, 42, 44, 45]. Yet, a cholinergic-hormonal interaction cannot explain the reduction of HPS and classically-conditioned analgesia [55] which are impervious to pituitary-adrenal manipulations [43, 53, 54, 56]. In contrast, Watkins and coworkers [55] propose cholinergic mediation in rostral cerebral areas for classically-conditioned analgesia, and the medullary nucleus raphe alatus or parabrachial region for HPS analgesia. The role of these regions in all other muscarinic-sensitive forms of stress-induced analgesia is not known. Further work is necessary to determine whether the cholinergic mediation of different forms of stress-induced analgesia is dependent upon: (a) a single locus of action, (b) separate neural loci, or (c) separate neural and hormonal loci.

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