Pharmacology Biochemistry and Behavior, Vol. 3, pp. 251–262. ANKHO International Inc., 1975. Printed in the U.S.A.

Cholinergic and Dopaminergic Blocking Agents Modulate Water Intake Elicited by Deprivation, Hypovolemia, Hypertonicity and Isoproterenol[1]

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(Received 15 June 1973)

BLOCK, M. L. AND A. E. FISHER. *Cholinergic and dopaminergic blocking agents modulate water intake elicited by deprivation, hypovolemia, hypertonicity, and isoproterenol.* PHARMAC. BIOCHEM. BEHAV. 3(2) 251–262, 1975. – In order to identify and differentiate separate components of an overall drinking system on neurochemical grounds, a few neuropharmacological blocking agents, already shown to affect the mediation of some thirst-related behaviors, were tested against a wide range of manipulations that elicit drinking behavior. Peripheral injections of scopolamine, an anticholinergic agent, or haloperidol, a catecholamine blocking agent with pronounced antidopaminergic actions, substantially reduced the water intake of rats induced to drink by periods of deprivations or by subcutaneous injections of either hypertonic saline, polyethylene glycol, or isoproterenol. When a combined injection of both scopolamine and haloperidol was given, hypovolemic and isoproterenol-induced drinking were almost entirely eliminated, but salt-aroused or deprivation-induced drinking were not totally abolished. In control studies, eating behavior elicited by either food deprivation or peripheral injection of 2-deoxy-d-glucose was not affected by these blocking agents. These experiments suggest that activation of cholinergic and dopaminergic neurons within central thirst-related systems are important physiological events underlying drinking behavior.

Scopolamine Haloperidol Central cholinergic mechanism Central dopaminergic mechanism
Deprivation-induced drinking Salt-aroused drinking Isoproterenol-induced drinking

ALTHOUGH significant progress has been made, the analysis of the brain system which monitors thirst related stimuli and then mediates the behavioral response of drinking remains incomplete. One promising approach has involved study of the effects of various central nervous system (CNS) manipulations on the drinking behavior of water-sated or water-deprived animals [28,38]. For example, the relatively selective changes in behavior produced by the central administration of cholinergic and anticholinergic drugs has provided evidence that the thirst-related behavior of rats is partially mediated by central cholinergic mechanisms [17, 26, 27, 36]. Other studies implicate dopaminergic and beta-adrenergic transmitters in thirst, as well [33, 50, 57].

Problems of thirst can also be analyzed by correlating changes in peripheral physiology with an organism's drinking behavior. The goal here is identification of the primary stimuli which cause animals to ingest water. Thus, alterations in both the osmotic concentration and volume of the extracellular body fluids (ECF) and, perhaps, increased circulating levels of the blood-borne hormone angiotensin, can contribute to the development of a central state which predisposes the animal to drink [20]. These physiological changes can be independently manipulated by various experimental procedures to evoke drinking behavior [14, 19, 54, 56].

Since such changes also occur during extended periods of water deprivation, the implication is strong that natural thirst may represent a summation of the input from these primary stimuli to separate receptor systems in the brain.

It thus becomes of interest to determine if thirst-related components which have been differentiated in terms of physiological events occurring in peripheral systems can be differentiated on the basis of central neurochemical events as well. If they can be, then tools may exist for testing whether water intake after deprivation indeed represents the summed output of neural systems coded to monitor hypovolemia, hypertonicity and hypotension, or whether a different conceptual model will be necessary. To these ends, several neuropharmacological blocking agents were

[1] Supported by National Institute of Mental Health Grant MH-1951 (To A.E.F.). M.L.B. was supported by a National Institute of Mental Health predoctoral traineeship, MH 11114.

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tested against a wide range of manipulations that elicit drinking. Specifically, these experiments examined the effects of scopolamine (an anticholinergic agent) and haloperidol (a catecholaminergic blocking agent) on the drinking behavior of rats challenged by various dipsogenic stimuli. The stimuli used to induce drinking were hypovolemia, hypertonicity, water deprivation and peripheral injections of the beta-agonist isoproterenol. In addition, a number of control studies involving eating tests were run to determine the behavioral selectivity of the drug effects observed.

GENERAL METHODOLOGY

Animals

Adult male, albino rats (Sprague-Dawley Strain; Zivic-Miller Lab., Pgh., Pa.) weighing between 350 and 450 g at the start of the experiments were housed in individual metal cages (14 in. × 6-1/2 × 6-1/2 in.) with wire mesh floors, located in a small colony room. Temperature was maintained at approximately 22°C and a 12-hour light–dark cycle (light: 7 a.m.-7 p.m.) was imposed for the duration of the experiments. Tap water and food pellets (Purina Chow) were available ad lib in the home cages until the day of testing.

Drugs

Chemicals used to induce drinking behavior. The following solutions were made up just prior to their injection:
Polyethylene glycol (PEG). A 30% solution of polyethylene glycol (Carbowax, compound 20-M; Union Carbide Corporation) dissolved in 0.15 M sodium chloride (w/w). The polyethylene glycol was dissolved by heating the mixture to approximately 50°C while it was being constantly stirred.
Hypertonic saline solution. A 2.0 Molar sodium chloride solution.
Isoproterenol Solution. 0.04 mg of DL-Isoproteronol HCL (Sigma Co.) dissolved in 1.0 cc of isotonic saline (0.15M).

Blocking agents. The following drugs were mixed or diluted with isotonic saline to the dosage required, placed in light-resistant glass bottles, and stored under refrigeration (0–10°C). New solutions were made up each month. Prior to their injection the drugs were warmed to room temperature. The dosages required were injected in volumes of 1.0 cc per kg of body weight.
Scopolamine hydrochloride. (M.W. = 339.8: Sigma Co.).
Haloperidol (M.W. = 375.9). In the form Haldol ® (McNeil laboratories: one cc ampoules containing 5.0 mg haloperidol, 0.5 mg methylparaben, 0.05 mg propylparaben, and lactic acid for pH adjustment to 3.4 ± 0.2).
Haloperidol vehicle solution. Contains 0.5 mg of methylparaben and 0.05 mg of propylparaben dissolved in 1.0 cc of 0.15 M NaCl with enough lactic acid added to adjust the pH to approximately 3.4.

Rationale for Blocking Agents

Anticholinergic Agent. Since drinking behavior in the rat seems to be mediated, at least in part, by central cholinergic mechanisms of the muscarinic type [17, 36, 52] an antimuscarinic agent (scopolamine) was chosen for use in these experiments.

On the basis of available data [13, 25, 34] the following doses of scopolamine hydrochloride (expressed as the salt) were selected for injection (IP) 1/2 hr before the drinking tests: 0.1, 0.5, and 1.0 mg per kilogram (kg) of body weight.

Dopaminergic Blocking Agent. Drinking elicited by central injections of angiotensin into the preoptic area of rats is not affected by pretreatment of that site with either anticholinergic agents or with alpha or beta adrenergic blocking agents [21,24]. However, angiotensin-induced drinking can be blocked with centrally administered haloperidol, a drug which can block both alpha adrenergic and dopaminergic receptors [21]. Since the alpha blocking agent phentolamine is ineffective, the effects of haloperidol may be due primarily to blockade of dopaminergic receptors. Moreover, this finding suggests that any drinking associated with high levels of circulating angiotensin involves selective action on a central dopaminergic system.

It is possible to indirectly test this suggestion with peripheral injections of haloperidol. At low dose levels subcutaneous (SC) injections of haloperidol selectively block or alter a variety of behavioral activities which are thought to be mediated by central dopaminergic systems [8, 16, 31, 32]. At higher doses, typical haloperidol-induced behavioral changes are more explicable in terms of the action of haloperidol on both central dopaminergic and noradrenergic neuronal mechanisms. Thus, low doses of haloperidol might be expected to selectively block dopaminergic receptors while leaving noradrenergic receptors relatively intact. Haloperidol, in the form Haldol ®, was injected sc 1 hour before a drinking test in one of the following doses (expressed as the base): 0.02, 0.08, or 0.17 mg per kg of body weight [30].

EXPERIMENT 1: DRINKING INDUCED BY HYPOVOLEMIA

In this experiment, rats were made hypovolemic by injections of a polyethylene glycol solution (PEG) [54] and the effects of the blocking agents on the subsequent increase in water intake were tested.

Procedure

Three days before testing, animals were removed from their home cage room and placed in test cages located in a room with identical temperature and lighting conditions. These test cages were similar to the home cages except that water was delivered via Plexiglas drinking wells affixed to the front of the cage and fed by 50 ml burettes. During the 3 days of acclimation, food pellets and water were available continuously. On the fourth day, food was removed at 9:00 a.m. and the water was removed at 10:00 a.m. This differential removal of food and water was done to minimize the likelihood that any drinking seen thereafter was due to oropharyngeal factors which accompany the eating of dry food (e.g., a relatively dry mouth). Immediately following the removal of water, the rat was etherized and injected subcutaneously in the back with 5 cc of a 30% solution of polyethylene glycol. The entire injection procedure took about 3 min to complete, after which the animal was returned to its test cage. Five or 5-1/2 hr later drug or placebo injections were given (see below), and 6 hr following the injection of PEG the drinking apparatus was returned to the cage. The amount of water consumed was recorded to the nearest 0.1 cc one hr later.

For the study on the effects of scopolamine 16 rats were randomly assigned to 4 equal groups. Each group received one of the 3 doses of scopolamine HCl or a placebo injection of isotonic saline in equivalent volumes 30 min before the drinking test. For the haloperidol treatment, 16 additional rats were randomly assigned to 4 equal groups. Each

TABLE 1

THE EFFECTS OF SCOPOLAMINE AND HALOPERIDOL ON HYPOVOLEMIC-INDUCED DRINKING†

Drug	Dose mg/kg	Dose μmole/kg‡	N	$\bar{X}$ Water Intake (±SE) in cc 1 hr	Change from Placebo
Isotonic Saline§	(placebo)		4	11.2 ± 1.7	–
Scopolamine HCl	0.10	0.29	4	11.3 ± 1.6	0%
	0.50	1.46	4	6.3 ± 1.3*	–45%
	1.00	2.93	4	6.6 ± 0.9*	–41%
Haloperidol vehicle	(placebo)		4	8.1 ± 1.0	–
Haloperidol	0.02	0.05	4	5.3 ± 0.2*	–35%
	0.08	0.21	4	2.6 ± 0.3*	–68%
	0.17	0.45	4	0.6 ± 0.3*	–93%
Scopolamine HCl§ +Haloperidol[a]	0.50 0.02	1.46 0.05	3	0.4 ± 0.4*	

*Significantly different from the placebo intakes at 0.05 level; one-tailed *t* test for unpaired observations

†5 cc of 30% polyethylene glycol solution injected SC 6 hr before access to water

‡Expressed as the base

§IP injections 30 min before drinking test

[a]SC injections 60 min before drinking test

group received one of the 3 doses of the neuroleptic drug or the haloperidol vehicle solution subcutaneously in the back 60 min before the drinking test. (The haloperidol vehicle solution was diluted with isotonic saline prior to injection so that the concentration of the bacteriostatic agents was comparable to that found in the 0.17 mg/kg dose of the haloperidol solution).

Results and Discussion

It seems evident that some of the drinking seen following PEG treatment is dependent upon cholinergic systems (Table 1). It is interesting to note that increasing the dose of scopolamine HCl beyond 0.5 mg/kg does not lead to a corresponding increase in the effectiveness of the anticholinergic agent to reduce water consumption.

Haloperidol appears to reduce water intake in a dose-response fashion; however, since the selectivity of the drug effect at the highest dose (0.17 mg/kg) is open to question (see Experiments 5 and 6), the dose-dependency of this effect remains to be firmly established.

A combined injection of scopolamine and haloperidol, in doses that reduced water intake by 45% and 35% respectively when each drug was given alone, completely blocked the drinking behavior of hypovolemic rats (Table 1). Combined injection of these blocking agents failed to reduce food intake (Experiments 5 and 6) and therefore indicates that the complete blockade of PEG-induced thirst was not due to a general depression of behavior. Moreover, the complete elimination of drinking obtained in this fashion suggests that hypovolemically-induced thirst is critically dependent upon the activity of both cholinergic and catecholaminergic mechanisms.

EXPERIMENT 2: SALT-AROUSED DRINKING

In this experiment cellular dehydration was induced by injecting a concentrated solution of hypertonic saline [56] and the effect of each neuropharmacological blocking agent on the resultant drinking responses was examined.

Procedure

The procedure was similar to that described in the previous experiment, except for the method of inducing water ingestion. These animals were injected SC with 2 cc of a 2M sodium chloride solution under ether anesthesia and the drinking apparatus was returned to the cage 1 hour later, for a 1 hr drinking test.

Thirty-two rats were randomly assigned and equally distributed among 8 different treatment groups. Four groups received IP injections of either isotonic saline, 0.1 mg, 0.5 mg, or 1.0 mg per kg body weight of scopolamine HCl 30 min before the animals had access to the drinking well. The 4 remaining groups were given SC injections of either haloperidol vehicle solution, 0.02 mg, 0.08 mg, or 0.17 mg per kg of haloperidol at the same time that the hypertonic NaCl solutions were injected, i.e., 60 min before the water was made available to the rats.

TABLE 2

THE EFFECTS OF SCOPOLAMINE AND HALOPERIDOL ON SALT-AROUSED DRINKING†

Drug	Dose (mg/kg)	N	$\bar{X}$ Water Intake (±SE) in cc 1 hr	Change from Placebo
Isotonic Saline‡	(placebo)	4	10.5 ± 0.7	–
Scopolamine HCl	0.10	4	10.5 ± 2.0	0%
	0.50	4	4.4 ± 1.6*	– 58%
	1.00	4	2.9 ± 1.3*	– 72%
Haloperidol vehicle	(placebo)	4	9.8 ± 0.4	–
Haloperidol	0.02	4	8.9 ± 1.0	– 9%
	0.08	4	3.8 ± 1.0*	– 62%
	0.17	4	0.0 ± 0.1*	–100%
Isotonic Saline‡ +Haldol Control§	(placebo)	3	8.7 ± 0.8	–
Scopolamine HCl +Haloperidol	0.50 0.08	3	3.3 ± 1.0*	– 62%

*Significantly different from the placebo intakes at 0.05 level; one-tailed *t* test for unpaired observations

†2 cc of a 2.0 M NaCl solution injected SC 60 min before access to water

‡IP injections 30 min before drinking test

§SC injections 60 min before drinking test

Results and Discussion

The effects of scopolamine and haloperidol on salt-aroused drinking are summarized in Table 2. Included in Table 2 is the result of a combined injection of scopolamine and haloperidol in doses which reduced water intake by 58 and 62 percent respectively when each was given alone. Both scopolamine and haloperidol reduced the intakes of rats responding to a hyperosmolar condition. The effects of scopolamine are similar to those reported by DeWied [15] using 1.2 ml of a 15% NaCl solution to induce drinking and atropine sulfate as the cholinergic blocking agent.

The results obtained in the present experiment suggest that the drinking response to hypertonicity may be mediated in part by both cholinergic and catecholaminergic mechanisms. However, the combined injection of scopolamine and haloperidol did not completely eliminate the water consumption of salt-aroused rats as it had in the tests with PEG-induced drinking. One possible explanation for this difference is that another neurochemical factor (or factors) is involved in the mediation of salt-aroused drinking, and that it is not totally dependent on the functional integrity of the neuronal systems blocked or depressed in this study. The administration of other classes of neuropharmacological agents which alter the endogenous activity of other neurochemicals would be one way of testing this possibility. In regard to this hypothesis, DeWied [15] failed to find any reduction in the drinking response of salt-aroused rats following ip injection of the antiserotonergic agent methysergide.

It is equally possible, however, that the difference between the effectiveness of a combined injection of the blocking agents on PEG-induced and salt-aroused drinking is due to the rather different physiological states of the organisms at the time of testing. Thus, differences in drug distribution and/or excretion under the two dipsogenic conditions, for example, may lead to a difference in drug efficacy and, therefore, partially account for the results obtained (see General Discussion).

EXPERIMENT 3: ISOPROTERENOL-INDUCED DRINKING

In the sated rat, a suitable dosage of isoproterenol will produce a number of effects, including hypotension, elevated plasma renin levels, and increased water intake [33,42]. This method of eliciting drinking behavior was used to test the effects of the blocking agents haloperidol and scopolamine on water intake associated with increased levels of circulating renin and/or angiotensin II.

Procedure.

Testing was done in the home cages. On a test day food was removed at 10:00 a.m.; one hr later the water was removed and the rat was injected SC in the back with 0.04 mg/kg of isoproterenol HCl. Sixty minutes following this injection tap water was made available to the rat via a metal water spout fed by a 50 ml burette. The amount of water consumed over the next hour was recorded.

Since the drinking response to isoproterenol often varied considerably from one animal to another, each rat was used

TABLE 3

THE EFFECTS OF SCOPOLAMINE AND HALOPERIDOL ON ISOPROTERENOL-INDUCED DRINKING†

Group	N	$\bar{X}$ 1-Hr Water Intake (± SE) in cc Following Injections of: Isotonic Saline	Haloperidol‡ (mg/kg) 0.02	0.08	0.17	Change from Placebo
IV	4	6.5 ± 1.7	5.4 ± 0.2*			–17%
V	4	9.5 ± 1.6		2.0 ± 0.7*		–79%
VI	4	6.5 ± 1.7			0.8 ± 0.5*	–88%
			Scopolamine HCl§ (mg/kg) 0.1	0.5	1.0	
I	4	9.6 ± 1.0	6.0 ± 2.2			–38%
II	4	7.9 ± 1.1		4.3 ± 1.9		–46%
III	4	8.4 ± 0.3			1.8 ± 1.0*	–79%
			Haloperidol (0.08 mg/kg) + Scopolamine HCl (0.5 mg/kg)			
II	4	7.9 ± 1.1		0.4 ± 0.3*		–96%

*Significantly different from placebo intakes at 0.05 level; one-tailed *t* test for paired observations
†0.04 mg/kg of Isoproterenol HCl injected SC 60 min before access to water
‡SC injections 60 min before drinking test
§IP injections 30 min before drinking test

as its own control. Twenty rats were randomly assigned and equally distributed among 5 groups. Each group was given a placebo injection of isotonic saline and one or more doses of the blocking agents in a counterbalanced order at one week intervals. (Pilot work showed that there was no difference between the amount of water consumed following isotonic saline and haloperidol vehicle injections.) Groups that received haloperidol treatment were injected 1 hr before the drinking test while the scopolamine treatment groups were injected 1/2 hr before the water was returned.

Results and Discussion.

Table 3 presents the results of the blocking agents on isoproterenol-induced drinking in a 1 hour test. The drinking induced in sated rats by peripheral injections of isoproterenol was reduced in a dose-response fashion by either scopolamine or haloperidol. However, as Table 3 indicates, there was considerable variability in response to the two lower doses of scopolamine.

The amounts of water consumed following the various doses of haloperidol were much more consistent within a particular treatment group. Combined injections of haloperidol (0.08 mg/kg) and scopolamine HCl (0.5 mg/kg) given just prior to the drinking test virtually eliminated isoproterenol-elicited drinking (Table 3).

It is of interest to note that the two experiments in which vascular volume was manipulated (PEG and isoproterenol injections), but not the one in which tonicity was being manipulated (hypertonic saline injections), give identical results with combined injections of haloperidol and scopolamine, namely, a complete abolishment of the drinking response. These data suggest that cholinergic and catecholaminergic systems may be crucial for the mediation of the drinking behavior induced by volemic changes.

Lehr *et al.* [33] have reported that isoproterenol-induced drinking is totally blocked by peripheral administration of a beta-adrenergic blocking agent. Recent evidence [18,29] suggests that this reduction in drinking by beta-blockers may be due to the direct blockade of the peripheral adrenergic receptors upon which isoproterenol appears to act. Since there is no evidence that either haloperidol or scopolamine (given in the manner employed in the present study) effects peripheral beta-adrenergic systems, we tentatively view the blocking effects obtained in the present experiment as being primarily a result of the disruption of central neurochemical systems that are involved in isoproterenol-induced drinking (see General Discussion).

EXPERIMENT 4: DEPRIVATION-INDUCED DRINKING

Procedure.

Twenty-eight animals were placed in individual test cages provided with metal water spouts fed by 50 ml burettes. After several days of adaptation, water was removed at 1

TABLE 4

THE EFFECTS OF SCOPOLAMINE AND HALOPERIDOL FOLLOWING 22-HR WATER DEPRIVATION†

Group‡	Drug	Dose (mg/kg)	N	$\bar{X}$ HOH Intake (±SE) in cc 1 hr	Change from Placebo
1	Isotonic Saline	(placebo)	4	16.1 ± 0.8	
	Haloperidol	0.02		16.4 ± 2.1	+ 2%
2	Isotonic Saline	(placebo)	4	19.8 ± 1.3	
	Haloperidol	0.08		9.6 ± 1.6*	–52%
3	Isotonic Saline	(placebo)	4	15.8 ± 2.9	
	Haloperidol	0.17		2.8 ± 0.9*	–82%
4	Isotonic Saline	(placebo)	4	16.4 ± 1.5	
	Scopolamine HCl	0.1		14.3 ± 0.6	–10%
5	Isotonic Saline	(placebo)	4	16.0 ± 1.2	
	Scopolamine HCl	0.5		10.1 ± 0.9*	–37%
6	Isotonic Saline	(placebo)	4	18.5 ± 1.5	
	Scopolamine HCl	1.0		8.9 ± 1.0*	–52%
7§	Isotonic Saline	(placebo)	7	19.1 ± 1.7	
	Haloperidol	0.08		4.7 ± 1.4*	–76%
	+Scopolamine HCl	0.5			

*Significantly different from placebo intake at 0.05 level; one-tailed *t* test for paired observations
†22 hr of water deprivation; food available ad lib
‡Groups 1–3: SC injections of placebo or drug 1 hr before test
Groups 4–6: IP injections of placebo or drug ½ hr before test
§Placebo and drug injections given 1 hr or ½ hr before test (as above)

p.m. for 22 hr. On the following day, the water tube was returned at 11:00 a.m. and the water intake for the next hour was recorded. This procedure was repeated at weekly intervals. The animals were randomly assigned among 7 treatment groups and each group (N = 4) was designated to receive a SC injection of isotonic saline (1.0 cc/kg) and one of the following treatments: 0.02 mg, 0.08 mg or 0.17 mg per kg of haloperidol, 0.1 mg, 0.5 mg or 1.0 mg/kg of scopolamine or a combined dose of 0.08 mg/kg haloperidol and 0.5 mg/kg scopolamine. On the third deprivation experience, half of the animals in each treatment group were given placebo injections of isotonic saline prior to the drinking hour and the other half received injections of a blocking agent. A week later, water was again removed for 22 hr and the injection procedure was reversed for each half of the treatment groups.

In a corollary experiment, the effectiveness of selected doses of scopolamine and/or haloperidol was tested following a shorter (11 hr) period of deprivation. Groups of 4 animals were deprived of water for 11 hr (10:00 a.m. to 9:00 p.m.) and injected with 0.5 mg/kg of scopolamine HCl, 0.08 mg/kg of haloperidol or both before a 1 hr drinking test. The water intakes under the drug conditions were compared to the amount of water consumed following isotonic saline injection (1.0 cc/kg).

Results and Discussion

The lowest dose of scopolamine or haloperidol was ineffective in altering the drinking response following 22 hr of deprivation but the higher doses significantly reduced water intake (see Table 4).

Combined injections of haloperidol and scopolamine (Group 7, Table 4) reduced the intakes of a majority of the 22 hr deprived animal to a greater degree than either drug given alone. Two of the rats in Group 7 did not drink any water; 3 rats showed reductions greater than 75 percent and the water intakes of the 2 other animals were reduced by about 50 percent. The results of the 11 hr deprivation studies are summarized in Table 5.

Haloperidol reduced water intake by the same percentage following 11 or 22 hr of deprivation, although the re-

TABLE 5

THE EFFECTS OF SCOPOLAMINE AND HALOPERIDOL FOLLOWING 11-HR WATER DEPRIVATION†

Group	Drug	Dose (mg/kg)	N	$\bar{X}$ HOH Intake (±SE) in cc 1 hr	Change from Placebo
8‡	Isotonic Saline	(placebo)	4	11.1 ± 1.6	
	Haloperidol	0.08		5.5 ± 0.5*	–51%
9§	Isotonic Saline	(placebo)	4	12.8 ± 2.3	
	Scopolamine HCl	0.5		3.2 ± 1.8*	–75%
10‡§	Isotonic Saline	(placebo)	4	11.8 ± 2.0	
	Haloperidol	0.08		2.5 ± 1.1*	–79%
	+Scopolamine HCl	0.5			

*Significantly different from placebo intakes at 0.05 level; one-tailed *t* test for paired observations
†11 hr of water deprivation; food available ad lib
‡SC injections 60 min before the drinking test
§IP injections 30 min before the drinking test

duction in volume drunk was twice as great at 22 hr (relative to the respective placebo conditions). Alternately, although the percent reduction in intake following scopolamine treatment was much greater at 11 hr than at 22 hr, there was no significant difference in the reduction in volume drunk across the two conditions. These distinctions are important in assessing these data (see General Discussion) since the intake following 22 hr of deprivation was of a different order of magnitude than was the case for any other challenge utilized in this study.

The effectiveness of scopolamine in these experiments is consistent with the numerous reports demonstrating partial blockade of deprivation-induced drinking by peripheral injections of antimuscarinic agents [27, 47, 52]. The effects of haloperidol, however, have not been reported in the literature and provide evidence that catecholaminergic systems may be involved in the mediation of drinking behavior following water deprivation.

The combined injections of scopolamine and haloperidol failed to block drinking completely and, in this respect, resembled the incomplete blocking action of the combined injection on salt-aroused drinking.

The testing of blocking agents against varying levels of deprivation deserves a more extensive investigation. It should be appreciated, however, that the same drug may be more, or less, effective when applied at different times of the day [44], and that the amount of water which a rat consumes after a deprivation period of less than 24 hr is a function of the time of day during which deprivation occurs [40]. Thus, circadian rhythms underlying both drug susceptibility and drinking patterns may be contributory factors to the outcomes of experiments dealing with the interaction of drugs and ingestive behaviors, and their possible influences must be carefully assessed before any firm conclusions can be drawn.

EXPERIMENT 5: DEPRIVATION-INDUCED EATING

The question to be dealt with in these experiments is whether the effects of haloperidol and scopolamine on water intake are selective with respect to drinking behavior or whether these agents non-selectively depress all ingestive behaviors.

Procedure

Naive adult, male rats weighing between 350 and 450 g were acclimated to test cages for 3 days. On the 4th day, food pellets were removed from the cages at 1:00 p.m. and returned 22 hr later. Each rat experienced this deprivation procedure twice, separated by 1 week, before any drug treatments were used. On the third deprivation experience, rats were injected with a drug or placebo solution prior to the eating test and the amount of food consumed in the hour following reintroduction of the food pellets was recorded to the nearest 0.1 g.

Six rats were given SC injections of a haloperidol vehicle solution and 0.08 mg/kg of haloperidol on separate occasions and in a counter-balanced order 1 hr before the eating test. In the same manner, another 3 rats received SC injections of haloperidol vehicle solution and 0.17 mg/kg of haloperidol. An additional group of animals (N = 3) were given IP injections of isotonic saline and 0.5 mg/kg of scopolamine HCl in a crossover design 30 minutes before the eating test.

One of the peripheral effects of a systemic injection of scopolamine is a reduction in salivery flow. Thus, a food deprived animal who had received an injection of this anticholinergic drug might not be able to eat appreciable amounts of dry food because of the excessive, unpalatable accumulation of dry food particles, even though the rat is hungry.

TABLE 6

THE EFFECTS OF SCOPOLAMINE AND HALIPERIDOL FOLLOWING 22-HOUR FOOD DEPRIVATION†

Drug‡	Dose (mg/kg)	N	$\bar{X}$ 1-Hour Food Intakes (±SE) in grams Dry Food	Wet Mash
Isotonic Saline	(placebo)	6	8.8 ± 0.6	
Haloperidol	0.08		8.2 ± 0.8	
Isotonic Saline	(placebo)	3	7.6 ± 1.1	
Haloperidol	0.17		0.7 ± 0.1*	
Isotonic Saline	(placebo)	3	8.4 ± 1.1	
Scopolamine HCl	0.5		3.1 ± 0.8*	
Isotonic Saline	(placebo)	6		21 ± 1.2
Scopolamine HCl	0.5			19 ± 2.6
	1.0			17 ± 2.3
Isotonic Saline	(placebo)	3		24 ± 0.6
Haloperidol	0.17			5 ± 2.3*
Isotonic Saline	(placebo)	6		24 ± 0.8
Scopolamine HCl	0.5			23 ± 1.9
+Haloperidol	0.08			

*Significantly different from placebo intakes at 0.05 level; one-tailed *t* test for paired observations

†22 Hours of food deprivation; water available ad lib

‡Haloperidol injected SC 1 hr before test; Scopolamine injected IP ½ hr before test; corresponding placebo injections given in an equivalent manner

In order to avoid this possible contaminating variable, a wet food was used in the following test. For three consecutive days 6 rats were given access to a mixture of powdered food (Purina Chow) and water (2 parts HOH: 1 part chow, w/w: mixed fresh daily) from nonspillable food cup placed into their cages. On the fourth day, a fresh wet mash mixture was placed into the cages at 11:00 a.m. and 2 hr later all foods, i.e., wet mash and food pellets, were removed for 22 hr. The next day, each animal received an IP injection of either isotonic saline (1.0 cc/kg), 0.5 mg/kg or 1.0 mg/kg of scopolamine HCl 30 min before the wet mash was introduced into the cage at 11:00 a.m. The amount of wet mash consumed over the next hour was recorded. This deprivation procedure was applied to all the animals on 3 occasions, separated by 5 day intervals, and on each occasion a rat was given a different treatment in an order which counter-balanced across animals.

Result

When either dry food or wet mash was available following 22 hr of deprivation the highest dose of haloperidol (0.17 mg/kg) substantially reduced food intake, while the intermediate dose (0.08 mg/kg) had no effect on the consumption of dry food (Table 6). The intermediate dose of scopolamine (0.5 mg/kg) did reduce the intake of dry food; however, neither this dose nor the next highest dose of scopolamine (1.0 mg/kg) had any effect on the consumption of wet mash, probably because the latter food eliminated the contaminating variables associated with the reduced salivary flow that follows an injection of an anticholinergic drug. Combined injections of haloperidol and scopolamine (in doses equivalent to those employed in the previous drinking experiments) did not affect intake of wet mash (Table 6).

EXPERIMENT 6: EATING INDUCED BY INJECTIONS OF 2-DEOXY-D-GLUCOSE

In Experiments 1, 2, and 3 the reductions in water intake by the blocking agents were based on drinking behaviors induced by injections of chemicals. Therefore, the most appropriate behavioral control for selectivity of drug action in these experiments is to test the effects of these blocking

TABLE 7

THE EFFECTS OF SCOPOLAMINE AND HALOPERIDOL ON 2-DEOXY-D-GLUCOSE INDUCED EATING†

Drug‡	Dose (mg/kg)	N	$\bar{X}$ 1-Hour Consumption of Wet Mash (±SE) in grams
Isotonic Saline	(placebo)	3	18 ± 2.9
Scopolamine HCl	0.5	3	17 ± 1.8
	1.0	3	14 ± 2.5
Isotonic Saline	(placebo	3	17 ± 0.9
Haloperidol	0.08	3	18 ± 2.2
	0.17	3	1 ± 0.6*
Scopolamine HCl	0.5	3	16 ± 1.4
+Haloperidol	0.08		

*Significantly different from placebo intake at 0.05 level; one-tailed *t* test for unpaired observations

†750 mg/kg of 2-deoxy-D-glucose injected IP 90 min before access to food

‡Haloperidol injected SC 1 hr before eating test; scopolamine injected ½ hr before test; and corresponding placebo injections were given in an equivalent manner

agents on eating behavior which is also induced by chemical injections. When 2-deoxy-D-glucose (a specific inhibitor of intracellular glucose utilization) is injected into sated rats they increase their food intake [47].

Animals. The rats from the previous eating test were also used in this experiment.

Drugs. A 2-Deoxy-D-glucose solution (2-DG) containing 750 mg of 2-Deoxy-D-glucose (Sigma Corp.) dissolved in one cc of distilled water was prepared just prior to its injection.

Procedure

At 10:00 a.m. on the test day, food pellets were removed from the test cages and the rats were given wet mash (see Experiment 5). One hour later the wet mash was removed from the cage and each rat was injected IP with 750 mg/kg of 2-DG. Twenty-one rats were then randomly assigned to 7 groups. At 11:30 a.m. three of these groups were given sc injections of either haloperidol vehicle solution, 0.08 mg/kg or 0.17 mg/kg of haloperidol. At 12 noon, another 3 groups received IP injections of either isotonic saline, 0.5 mg/kg, or 1.0 mg/kg of scopolamine HCl. The remaining group received injections of 0.08 mg/kg of haloperidol 1 hr before the eating test and 0.5 mg/kg of scopolamine HCl 1/2 hr before the test. A fresh mixture of wet mash was returned to all the cages at 12:30 p.m. and 1 hr later the amount of food consumed was recorded to the nearest gram.

Results

Except for the highest dose of haloperidol, peripheral administration of haloperidol and scopolamine, alone or in combination, had no demonstrable effects on food intake elicited in sated rats by injections of 2-DG (Table 7).

Discussion

The data obtained in Experiments 5 and 6 suggest that except at the highest dose of haloperidol, for which tranquilizing effects were evident, the drug effects observed in the previous experiments are relatively selective with regards to the drinking behavior evoked by the various dipsogenic procedures and are probably not due to a general depression of behavior.

There is the intriguing possibility that the differential effects of scopolamine and haloperidol on food and water intake might be due to the ability of these drugs to differentially block the motor aspects of these ingestive responses. Haloperidol, for example, might block the licking movements used by rats to obtain water, but not affect the chewing movements used in ingesting food. Results obtained in a preliminary study using a liquid food indicate that this is an unlikely explanation. Four rats were offered a salty Metrecal solution after a 22 hr food deprivation period. Water was available ad lib. When rats are deprived of food for 24 hr they prefer this Metrecal solution to water, but the preference is reversed following a 24 hr period of water deprivation [37]. At weekly intervals, these rats were given peripheral injections of isotonic saline (1.0 cc/kg), haloperidol (0.08 mg/kg), or scopolamine HCl (0.5 mg/kg) just prior to the introduction of this liquid food following a 22 hr food deprivation period (in a manner identical to that described in Experiment 6). In a one hour eating test, the amounts of Metrecal ingested following isotonic saline ($\bar{X}$ = 21.2 ml), haloperidol ($\bar{X}$ = 18.5), and scopolamine ($\bar{X}$ = 21.5 ml) injections were not significantly different from each other.

GENERAL DISCUSSION

The results of this study suggest the pervasive involvement of cholinergic and catecholaminergic systems in the

several types of thirst investigated. Thus, the functional integrity of cholinergic and catecholaminergic (dopaminergic?) neurons within thirst-related systems seems to be important for the mediation of drinking behavior in the rat. While the antidipsogenic actions of scopolamine and haloperidol, given singly or in combination, could be due to a general behavioral depression, this possibility seems unlikely for two reasons. First, equivalent doses of these blocking agents did not effect eating behavior (except at the highest dose of haloperidol), and second, true sedative-hypnotic agents (e.g., barbiturates) will actually increase the drinking behavior of rats [35, 46].

The present data also suggest that a combined dose of scopolamine and haloperidol is less effective in reducing the water intake of salt-aroused and deprived rats than it is for animals drinking in response to PEG or isoproterenol. Thus, drinking induced by hypovolemia, or by simulation of a hypovolemic state, may be primarily dependent upon cholinergic and catecholaminergic neurons, while drinking associated with hypertonicity may involve an additional neurochemical factor, or factors. Other interpretations are also possible and because of the small sample size this suggestion should be viewed at this time as a working hypothesis worth further experimental study.

We suggest that the putative neurotransmitters whose activities were reduced by the blocking agents carry out their major thirst-related roles within the CNS. While it is not possible to completely rule out peripheral actions of scopolamine, there is good evidence that (at the doses used in this study) scopolamine's effects on various other behavioral events (and also on electrophysiological phenomena within the CNS) are due primarily to its central cholinolytic action [34]. It has been reported that intraperitoneal injections of the quarternary cholinergic blocking agent methylscopolamine bromide, which presumable does not cross the Blood-Brain-Barrier (BBB), reduces water intake of 23 hr water-deprived rats by 13–33 percent [23]. These results have been interpreted as evidence for a peripheral cholinergic component in "thirst-induced water consumption" [23]. There are a number of problems with this interpretation. First, there is no evidence provided that the effects of the quarternary cholinergic blocking agent (methylscopolamine) were selective for drinking behavior. More importantly, a brain organ which lies outside the BBB, the subfornical organ, contains cholinergic synapses [1] and is reputed to play a role in the central regulation of fluid balance in general [41], and drinking behavior in particular [45].

With respect to the site of action of haloperidol, there is no evidence that this neuroleptic agent, except in large doses, has a significant influence on peripheral sympathetic or parasympathetic function, or directly affects the cardiovascular system [11]. On the other hand, there are studies which strongly indicate that low doses of haloperidol (such as those employed in the present study) effect a variety of noningestive behaviors by disrupting central catecholaminergic systems, most likely of the dopaminergic kind [8, 16, 31, 32]. Coupled with the recent reports that directly implicate central dopaminergic systems in the control of drinking behavior [50, 55, 57], the cumulative evidence suggests that haloperidol's mode of action is probably the blocking of catecholaminergic receptors located within the CNS. Nevertheless, more experimental work will be necessary to differentiate clearly between central and peripheral effects of the blocking agents used in these studies. In a series of pilot studies we have applied micro quantities (5 μg) of haloperidol and scopolamine to central thirst-related sites following specific thirst challenges [6]. Preliminary findings indicate levels of blockade equivalent to those reported here following peripheral injections and strongly support the concept of central action.

Some cautionary notes are in order, however. It may not prove possible with these approaches alone to realistically assess the relative contribution of specific neurochemical mediators to the drinking behavior associated with the states of hypovolemia, hyperosmolarity and/or elevated levels of circulating renin-angiotensin. Differences in the physiological state of rats subjected to different kinds of thirst-inducing manipulations could provide the basis for the differential effectiveness of a particular pharmacological agent across dipsogenic challenges. For example, the hyperosmotic state of a salt-aroused rat may temporarily change the permeability characteristics of the BBB [43,53] and thereby alter the ability of a peripherally injected drug to penetrate into functionally important brain sites.

At another conceptual level, the differential effectiveness of a particular blocking agent across a number of dipsogenic conditions might be attributable to a failure to equate such conditions on motivational grounds. Thus, even though we attempted to equate amount of water ingested across most of the challenges utilized in these studies, more sophisticated motivational measures might uncover underlying differences in motivation which were in part responsible for the level of effectiveness of a blocking agent under a given condition.

In spite of such real or potential problems, the approach utilized here can yield important information and clues for further study. First, these data weaken substantially the hypothesis that differentiable components within an overall central thirst system have exclusive use of or are selectively or fully dependent upon any one of the transmitters under analysis. Although such results are initially disheartening, they provide an incentive to seek for alternative conceptual models. For example, one must be intrigued by the fact that all forms or levels of thirst we have investigated are partially blocked by systemic levels of anticholinergics which show no comparable effect on the food intake of hungry animals. Yet only one dipsogenic challenge, cholinergic central stimulation itself, is totally blocked by such anticholinergic action [18].

It is even more intriguing that the anticholinergic blockade of water intake following most thirst challenges is not only partial, but relatively constant. That is, the absolute reduction in expected water intake across many such challenges is similar enough to suggest a rather fixed contribution of a central cholinergic system to the maintenance or facilitation of quite differently derived states of thirst. Even when a particular challenge has been applied to different degrees, a given dose of an anticholinergic produces an almost constant decrement in the volume the animal drinks. In support of these statements, we would point out that when the same dose of scopolamine was given to rats deprived of water for 11 versus 22 hr (Tables 4 and 5), the absolute reduction in cc's of intake, although different, was not significantly different across the two conditions ($p<0.10$).

In a previous study in which central atropine was given to 6 and 23 hr deprived animals [7] we found even stronger evidence of a constancy in the absolute reduction in water intake (5.9 cc vs. 7.8 cc), although percent reduction was of

course very different (65 vs. 35 percent), due to the markedly different levels of total intake induced under the two conditions. We have also observed compelling evidence for a relatively fixed contribution of cholinergic mechanisms to the water ingestion induced by the more specific thirst challenges. Animals subjected to either osmotic or hypovolemic challenges (Tables 1 and 2, see also [7,18], showed virtually the same absolute decrease in water intake following a given dose of an anticholinergic as was seen under differing levels of deprivation.

On the basis of these results, we postulate that cholinergic neurons impinge on a number of thirst-related neural substrates, facilitating their responsivity in relatively equal measure, but being crucial to none. Such input would be readily susceptible to complete blockade by anticholinergics in non-challenged animals. Volemic and/or osmotic thirst challenges, however, would also activate non-cholinergic substrates on which cholinergic facilitatory pathways impinge, and anticholinergic intervention would suppress only the drinking attributable to the cholinergic facilitatory influence.

Our evidence suggests a similar ubiquity of function for dopamine pathways related to thirst, with further work necessary to differentiate the roles played by cholinergic and catecholaminergic neurons.

None of our data as yet implicate any single transmitter as crucial to or selectively linked to a specific thirst substrate or challenge.

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