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Drinking Induced by Parenteral Injections of Pilocarpine [1]

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GAY, P. E., S. C. BENNER, AND R. C. LEAF. *Drinking induced by parenteral injections of pilocarpine.* PHARMAC. BIOCHEM. BEHAV. 5(6) 633–638, 1976. – Parenteral (IP) injections of pilocarpine, in doses from 3.75–30 mg/kg, reliably produced drinking in water-satiated rats. This effect was not diminished by pretreatment with either centrally active (scopolamine, atropine) or peripherally active (methyl scopolamine, methyl atropine) cholinergic blocking agents, suggesting that pilocarpine does not induce drinking via a cholinergic mechanism. Repeated injections of low doses, but not high doses, of pilocarpine augmented drinking over trials.

Drinking Pilocarpine Scopolamine Atropine

IN PREVIOUS studies on the effects of pilocarpine on mouse killing by rats [1,2], it was noted that pilocarpine-treated animals often drank when water bottles were replaced following mouse killing tests. When water consumption was measured in the course of another mouse killing study [3], animals with a history of repeated pilocarpine injections drank significantly more water in a 1-hr test, when 1-hr water deprived and administered 7.5 mg/kg pilocarpine, than did similarly deprived vehicle-injected controls. Animals injected with 15 mg/kg pilocarpine, however, did not increase intake over control levels and 24-hr water deprivation decreased water intake in both pilocarpine dose groups.

Because pilocarpine is a cholinomimetic drug [5] and because central application of cholinergic substances can produce drinking (see review by Myers, [6]), it seemed possible that parenteral injections of pilocarpine might induce drinking by stimulation of cholinergic brain systems involved in drinking. On the other hand, pilocarpine also activates the parasympathetic system, producing water loss by salivation, lacrimation, defecation, and urination. This latter, peripheral action of pilocarpine might also provide a mechanism for thirst induction.

The purpose of the following experiments was two-fold: (1) to explore further the relationship between parenteral pilocarpine administration and water consumption, and (2) to determine whether the drinking resulting from pilocarpine administration is related to the cholinomimetic action of the drug.

EXPERIMENT 1

Previous work [3] suggested that, with short (1-hr) drinking periods, only low doses of pilocarpine elicited drinking in nondeprived rats. It seemed possible that a longer drinking period might identify a more dose-related function.

Animals

The animals were 30 adult male Holtzman rats (285–374 g). They were housed and tested in single Wahmann laboratory cages.

Procedure

All animals were adapted to a food and water schedule for 3 days in which water was continuously available from water tubes (graduated cylinders stoppered with rubber and a stainless steel drinking tube), but food (Purina pelleted chow) was removed for 3 hours each day. At the end of the 3-day adaptation period, animals were randomly assigned to 5 independent drug dose groups of 6 rats each. On Day 4, each group of rats received IP injections of 0.0, 3.75, 7.5, 15.0, or 30.0 mg/kg pilocarpine HCl (in 1 cc/kg 0.9% NaCl) at the beginning of the 3-hr drinking period. Water intake was measured every 15 min for the first hr and every 30 min thereafter. All tests were administered between 10 a.m. and 1 p.m.

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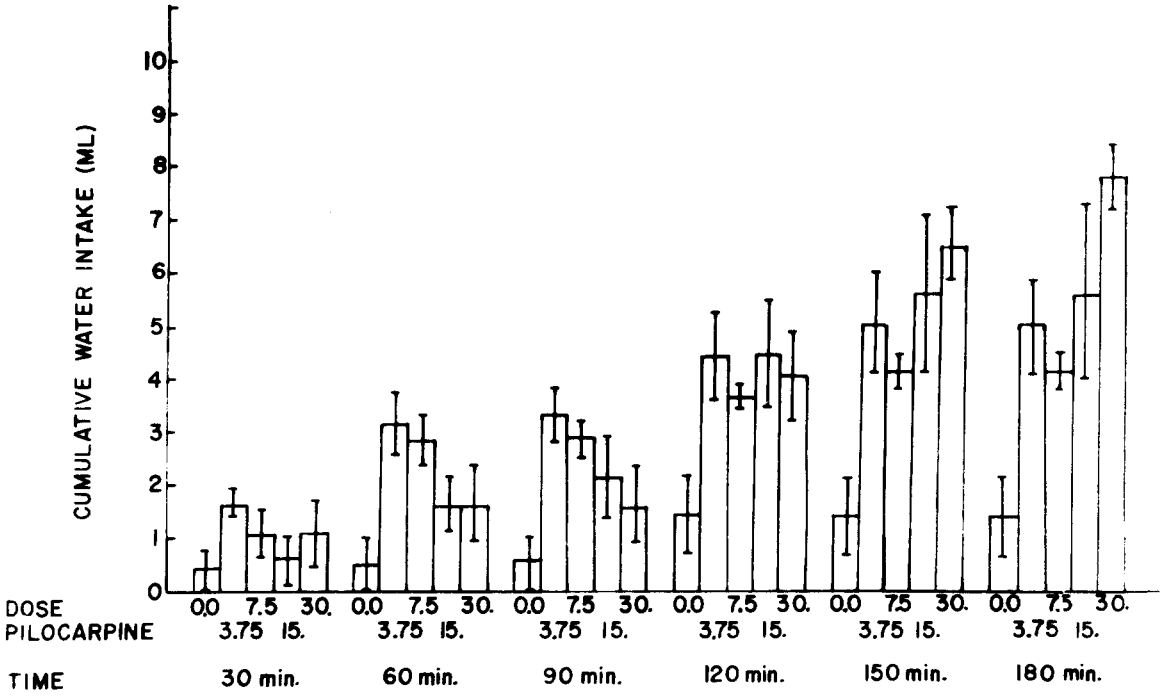

FIG. 1. Cumulative water intake as a function of 5 doses of pilocarpine and time postinjection.

Results and Discussion

Figure 1 shows cumulative water intake as a function of dose and time postinjection. Because the 15 min and 45 min readings did not differ from the general trend of the data, these data were omitted. The data for each time period were analyzed separately using analysis of variance followed by the following orthogonal comparisons: (1) vehicle control versus pilocarpine dose groups combined, and (2) low doses of pilocarpine (3.75 and 7.5 mg/kg) versus high doses of pilocarpine (15 and 30 mg/kg). On the basis of previous data [3], it was predicted that the pilocarpine groups would differ from the vehicle control and that there might be a time-dependent difference in the effects of the low and high dose groups.

As predicted, the pilocarpine-treated animals drank significantly more than the vehicle-injected controls. At 30 min postinjection, this difference first became statistically detectable (drug dose effect, $F(4,25) = 3.15$, $p<0.05$, orthogonal comparison vehicle vs pilocarpine dose groups combined, $F(1,25) = 7.15$, $p<0.05$) and the increased drinking only became more pronounced during successive time periods (drug dose effect, 180 min, $F(4,25) = 6.52$, $p<0.01$; same orthogonal comparison, $F(1,25) = 16.85$, $p<0.01$).

The time course for drinking was dose-related. While vehicle-treated animals drank little and usually only at the beginning of the test session, animals treated with low doses of pilocarpine drank most during the earlier segments of the drinking period and by 60–90 min postinjection, the cumulative milliliters consumed was inversely related to pilocarpine dose (orthogonal comparison low vs high dose, $F(1,25) = 7.20$, $p<0.05$). With higher doses of pilocarpine, however, drinking was delayed until later in the drinking period, so that by 180 min postinjection, total consumption was more directly related to drug dose (orthogonal comparison, low vs high dose, $F(1,25) = 6.42$, $p<0.05$). Observations of the animals suggested that drinking occurred only after the peripheral effects of pilocarpine injections had subsided.

EXPERIMENT 2

In Experiment 1, water consumption for the 7.5 mg/kg group was not as high as that observed previously [3]. However, the earlier study used rats with a history of repeated pilocarpine injections. Moreover, it is established that except at high doses, repeated injections of pilocarpine are necessary to reliably induce mouse killing by rats [2, 7, 8]. The following experiment explored the possibility that repeated injections of pilocarpine would increase water consumption.

In addition, this experiment had as a second purpose exploration of the duration and nature of the pilocarpine effect. For one-half of the animals the drinking period was extended to 4 hr. The remaining animals were given a 1-hr drinking period commencing 3 hr after pilocarpine administration.

Animals

The animals were 36 adult, male Holtzman rats (300–576 g). They were housed as in Experiment 1.

Procedure

At the beginning of the experiment, rats were randomly assigned to two groups. One group (N = 18) was never water deprived (immediate access group), while the other group was deprived of water for 3 hr daily (delayed access group).

All animals were adapted to a food and water schedule for 3 days in which water was available as determined by group assignment, but food was removed for 4 hr each day.

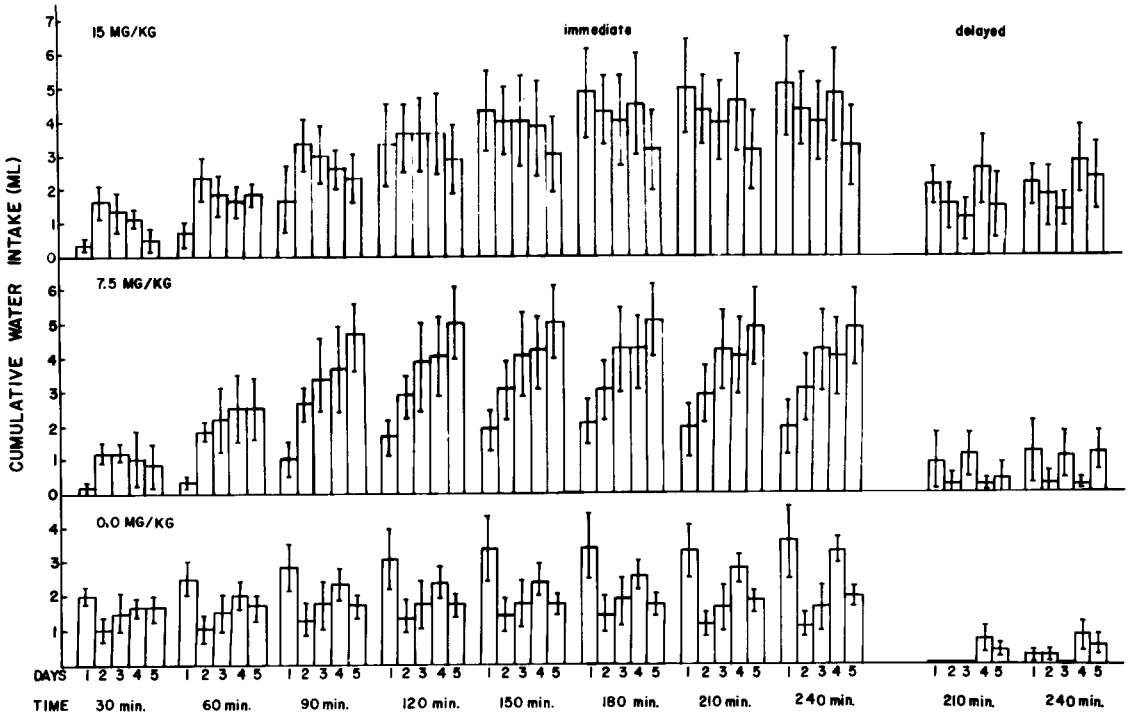

FIG. 2. Cumulative water intake as a function of 5 treatments with pilocarpine. Functions on the left are for animals which had the opportunity to drink immediately following pilocarpine injection. Functions on the right are for animals which were not allowed access to water until 3 hr after injection.

At the end of the 3-day adaptation period, animals in each access group were randomly assigned to 3 independent drug dose conditions (0.0, 7.5, or 15 mg/kg pilocarpine) of 6 rats each. On Days 4–8, rats received IP injections of the appropriate drug dose at the beginning of the 4-hr test period. For animals in the immediate access group, water was available immediately following injection and intake was measured every 30 min. For animals in the delayed access group, water was made available 3 hr after injection and intake was measured every 30 min for 1 hr. All tests were administered between 9:30 a.m. and 1:30 p.m.

Results and Discussion

Analysis of variance performed on the total amount of water consumed identified significant dose, $F(2,30) = 3.96$, $p<0.05$, and access group, $F(1,30) = 19.09$, $p<0.01$, effects as well as significant trials × dose, $F(8,120) = 2.11$, $p<0.05$, and trials × dose × access group, $F(8,120) = 2.14$, $p<0.01$, interactions. As in Experiment 1, pilocarpine injections increased drinking. The immediate access group drank significantly more water than the delayed access group, although the 1-hr access period allowed was more than adequate to drink the average 4–5 ml of water consumed by the immediate access group (see Fig. 2).

To further explicate the interactions, the data were analyzed separately for each access group. Analysis of variance performed on the delayed access group data identified only a significant drug dose effect, $F(2,15) = 4.62$, $p<0.05$. Thus, while delaying access to water for 3 hr following pilocarpine injection decreases water consumption substantially (from approximately 5 ml to 1 ml), a small dose effect can still be identified. At the same time postinjection, the immediate access group was demonstrating nearly zero consumption. This suggests that pilocarpine has, at most, a weak pharmacological action at 3–4 hr postinjection and that water loss, due to the peripheral effects of pilocarpine, may contribute to a minor extent to the increased water consumption seen in the delayed access group.

While none of the delayed access groups showed significant changes in water consumption over trials, there was such a change in one immediate access group (trials × drug dose interaction: $F(8,15) = 2.82$ $p<0.01$). The 7.5 mg/kg pilocarpine group increased water intake over trials, but intake remained relatively constant for the 15 mg/kg group. This is similar to the phenomenon of pilocarpine-induced mouse killing where, particularly at lower doses, repeated injections are necessary before killing occurs [2, 7, 8].

Over trials, maximum rate of intake appeared to shift to earlier segments of the drinking period (see Fig. 2). Ratings of the severity of side effects indicated that the peripheral effects of pilocarpine also decreased in duration with successive injections and that maximum drinking occurred following cessation of these peripheral effects. Thus, the malaise produced by pilocarpine may be inhibitory to drinking behavior.

EXPERIMENT 3

Experiment 2 suggested that water loss due to the peripheral effects of pilocarpine might contribute, at least to a minor extent, to the drug's action on drinking. Observations on the relationship between the side effects of pilocarpine and onset of drinking, however, suggested that the peripheral effects might inhibit drinking. The following experiment explored the effects of the cholinergic blocking agents, scopolamine and methyl scopolamine, on pilocarpine-induced drinking. It was hoped that by these means

we could evaluate the relative contribution of the central and peripheral effects of pilocarpine to pilocarpine-induced drinking.

Animals

The animals were 180 adult male Holtzman rats (300–500 g). They were housed as in Experiment 1.

Procedure

All animals were adapted to a food and water schedule for 2–3 days as in Experiment 1. At the end of the adaptation period, animals were randomly assigned to one of 6 IP pretreatment conditions (handling, saline vehicle, 0.5 mg/kg scopolamine HBr, 0.5 mg/kg scopolamine methyl Br, 1.0 mg/kg scopolamine HBr, or 1.0 mg/kg scopolamine methyl Br) and one of 5 IP doses of pilocarpine (0.0, 3.75, 7.5, 15.0, or 30.0 mg/kg) to create 30 independent groups of 6 animals each. On the following day, each animal was injected with the appropriate pretreatment drug (the handling group received an injection of zero volume because preliminary results suggested that volume of pretreatment agent might be an important factor), followed 15 min later by the appropriate dose of pilocarpine. The 3-hr drinking test began immediately after the pilocarpine injection. All tests were administered between 10 a.m. and 1 p.m.

Results and Discussion

Neither scopolamine nor methyl scopolamine reduced total water consumption (see Fig. 3), and, in fact, both doses of scopolamine appeared to facilitate such drinking. Analysis of variance performed on the total intake data identified a significant pretreatment effect, $F(5,150) = 4.16$, $p<0.01$, and a pilocarpine dose effect, $F(4,150) = 11.39$, $p<0.01$, but no interaction. *T*-tests demonstrated that both doses of scopolamine increased drinking beyond

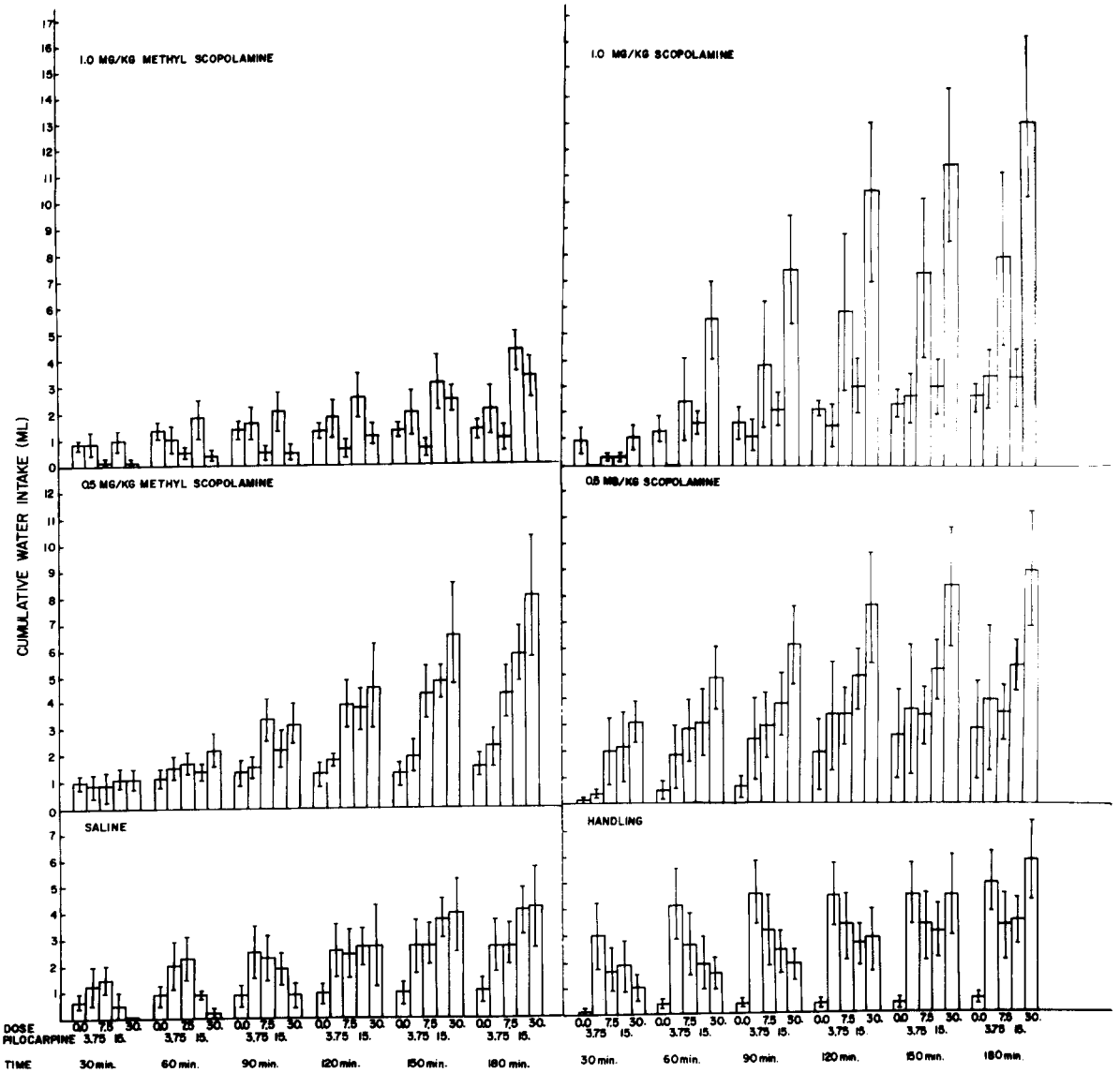

FIG. 3. Effects of pretreatment with scopolamine or methyl scopolamine on pilocarpine-induced drinking.

control levels. While pretreatment with the quaternary scopolamine analogue did not significantly enhance drinking, neither was a decrement in pilocarpine drinking observed.

Both pretreatment drugs also abolished the inverse dose-response relationship of pilocarpine to cumulative water intake usually seen at 90 min postinjection. Analysis of the cumulative intake at 90 min identified a significant pretreatment effect, $F(5,150) = 3.34$, $p<0.01$, a significant pilocarpine dose effect, $F(4,150) = 4.26$, $p<0.01$, and a significant pretreatment × pilocarpine dose interaction, $F(20,150) = 2.16$, $p<0.01$. As in the case of the 180 min data, *t*-tests indicated that scopolamine sigmificantly enhanced ($p<0.01$) drinking beyond control levels, but methyl scopolamine did not. These data suggest that the inverse dose-response function seen 90 min postpilocarpine is due to an inhibitory effect of the parasympathomimetic actions of pilocarpine. When these side effects are blocked, drinking becomes more dose-related. Moreover, these data suggest that pilocarpine-induced drinking is not totally due to water loss generated by the parasympathomimetic effects of pilocarpine.

EXPERIMENT 4

To test the hypothesis that the results of Experiment 3 were due to scopolamine's cholinergic blocking properties on sites involved in drinking and not to some other action of the drug (*e.g.*, general activation), an attempt was made to partially replicate that experiment using atropine and methyl atropine as the cholinergic blocking agents.

Animals

The animals were 29 adult, male Holtzman rats (280–331 g).

Procedure

The animals were adapted to the food and water schedule for 3 days as in Experiments 1 and 3. They were then randomly assigned to 3 pretreatment groups: 2.0 mg/kg atropine SO_4 (N = 8), 2.0 mg/kg atropine methylnitrate (N = 8), and saline vehicle (N = 8). (All doses of atropine are expressed as mg/kg of the base.) The next day, all animals were injected with the appropriate pretreatment drug, followed 15 min later by an IP injection of 30 mg/kg pilocarpine. A fourth group (N = 5) received 2.0 mg/kg atropine and vehicle. Drinking test were conducted as in Experiment 3.

Results and Discussion

Atropine and methyl atropine also failed to block pilocarpine-induced drinking (see Fig. 4). Animals in the atropine pretreatment group had a mean total intake similar to that of the methyl atropine pretreatment group and the saline pretreatment group. The combined pilocarpine groups, however, did drink significantly more than the group which received atropine and saline, but no pilocarpine (orthogonal comparison: $F(1,25) = 5.64$, $p<0.05$).

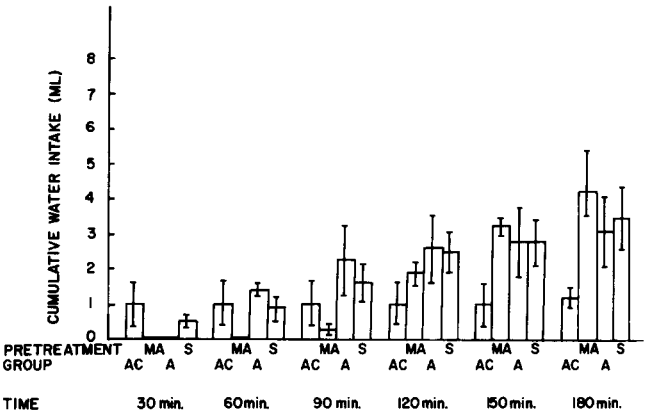

FIG. 4. Effects of pretreatment with atropine (A), methyl atropine (MA) or saline (S) on drinking induced by 30 mg/kg pilocarpine. The atropine control (AC) group received atropine and saline, but no pilocarpine.

GENERAL DISCUSSION

In these experiments, drinking was reliably produced by intraperitoneal injections of pilocarpine. Mean total consumption (in a 3-hr period) ranged from approximately 3.5–7 ml for the highest dose group. The time course of drinking was dose related, with animals receiving low doses of pilocarpine initiating drinking earlier in the test session than those receiving higher doses, although high doses eventually produced more drinking than low doses. Initiation of drinking seemed related to the disappearance of peripheral drug effects. Moreover, blocking these effects (Experiment 3) resulted in earlier initiation of drinking in animals receiving high pilocarpine doses.

Increased water consumption following pilocarpine does not appear to be a result of the drug's cholinomimetic activity. The peripheral parasympathetic effects of the drug seem to be primarily inhibitory to drinking. Blocking these effects enhances drinking and does not reduce it as would be expected if the drinking were due to water loss or a peripheral cholinergic thirst mechanism [4]. Blockade of both the central and peripheral effects of the drug also does not reduce drinking, suggesting that the drug is not acting directly on cholinergic brain systems for drinking. The mechanism by which pilocarpine does induce drinking remains unclear, although previous studies [3] demonstrating that amygdala lesions block such drinking suggest that the effect may be a central one.

While scopolamine significantly enhanced pilocarpine-induced drinking in Experiment 3, atropine failed to do so in Experiment 4. This suggests that the augmentation of drinking by scopolamine was due to some action of the drug other than its cholinergic blocking properties.

As for mouse killing, repeated administration of low doses of pilocarpine appeared to increase drinking behavior. This action may be due to habituation to peripheral effects of the drug [2,8] or possibly to the sensitization of some site involved in pilocarpine-induced drinking [7]. Further research is necessary to elucidate this issue and others concerning pilocarpine-induced drinking.

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