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Alcohol Intake of P Rats Is Regulated by Muscarinic Receptors in the Pedunculopontine Nucleus and VTA

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KATNER, S. N., W. J. MCBRIDE, L. LUMENG, T.-K. LI AND J. M. MURPHY. *Alcohol intake of P rats is regulated by muscarinic receptors in the pedunculopontine nucleus and VTA*. PHARMACOL BIOCHEM BEHAV **58**(2) 497–504, 1997.—Experiments were conducted to determine whether muscarinic receptors within the pedunculopontine nucleus (PPN) and ventral tegmental area (VTA) are involved in regulating ethanol drinking behavior in the alcohol-preferring P line of rats. Female P rats were given limited access (2 h/day) to 10% (v/v) ethanol and 0.0125% (g/100 ml) saccharin solutions. Food was available ad libitum. Cholinergic agents were microinjected unilaterally into the PPN or VTA immediately prior to ethanol access. Intra-PPN carbachol (1–4 μg/0.5μl), which can inhibit cholinergic neuronal activity within the PPN, decreased ethanol (70% decrease at the highest dose; $p < 0.05$) and saccharin (90% decrease at the highest dose; $p < 0.05$) intake in a dose-dependent manner within the first 30 min. Intra-PPN scopolamine (5–15 μg/0.5 μl), which can stimulate cholinergic neuronal activity within the PPN, decreased ethanol intake in a dose-dependent manner within the first 30 min (65% decrease at the highest dose; $p < 0.05$) without reducing saccharin intake. Intra-VTA methylscopolamine (1–10 μg/0.5 μl), a muscarinic antagonist, significantly ($p < 0.05$) reduced ethanol (60% decrease at the highest dose) and saccharin (50% decrease at the highest dose) intakes during the 2-h access period. Intra-VTA carbachol, a cholinergic agonist (1 and 2 μg/0.5 μl) decreased ethanol consumption in a dose-dependent manner within the first 60 min (50% decrease at the highest dose) without reducing saccharin intake. Overall, these results support an involvement of the cholinergic PPN–VTA system in regulating alcohol drinking and general consummatory behaviors of the P line of rats. © 1997 Elsevier Science Inc.

Pedunculopontine nucleus Ventral tegmental area Alcohol-preferring rats Carbachol Scopolamine Alcohol drinking Microinjection

CHOLINERGIC neurons (17,24), originating in the ventral portion of the pedunculopontine nucleus (PPN) (2,10) and laterodorsal tegmental nuclei, innervate the dopamine-rich ventral tegmental area (VTA) and substantia nigra (3,9,12,20, 25,33). A portion of the PPN input to the VTA appears to regulate neuronal activity via muscarinic receptors (7,16,18,29). The interactions of the PPN and VTA may play a key role in the complex brain circuitry that mediates reward. Microinjection of the muscarinic agonist carbachol into the PPN (36) or the muscarinic antagonist scopolamine into the VTA (14) increases the threshold for intracranial self-stimulation (ICSS) in the hypothalamus. The fact that both agents produced similar effects on the ICSS suggests that inhibition of the PPN cholinergic projection to the VTA may underlie this increased ICSS threshold because activating muscarinic receptors in the PPN reduce cholinergic neuronal activity (17), whereas blocking muscarinic receptors in the VTA prevent the excitatory input of cholinergic projections mediated by muscarinic receptors (16). These results are supported by the findings that, in a conditioned place preference (CPP) test, rats preferred

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compartments in which they received carbachol microinjected into the VTA (34). Furthermore, PPN lesions blocked CPP to morphine and amphetamine in naive rats (1), suggesting that the PPN may be involved in mediating the rewarding properties of these two drugs of abuse.

The important link mediating actions of cholinergic neurons from the PPN on reinforcement is most likely the mesolimbic dopamine (DA) projection from the VTA to the nucleus accumbens, which appears to be a critical part of the common pathway involved in the rewarding properties of drugs of abuse, including alcohol (8,15). In vivo microdialysis studies have shown that intraperitoneal (IP) ethanol administration (8), local perfusion with ethanol (37) and oral alcohol self-administration (31) stimulated DA release in the rat nucleus accumbens. Muscarinic cholinergic systems in the central nervous system (CNS) may also be involved in regulating alcohol intake. The muscarinic antagonists scopolamine and atropine, given subcutaneously (SC), decreased ethanol intake in the alcohol-preferring P line of rats (23) and in Sprague-Dawley rats (27). Because of the possible involvement of cholinergic systems in alcohol intake and the apparent role of cholinergic projections from the PPN in the regulation of DA systems in the VTA and in brain reward processes, the present study was undertaken to examine the involvement of muscarinic receptors within the PPN and VTA in mediating alcohol drinking behavior of the alcohol-preferring P line of rats.

METHODS

Adult female rats from the alcohol-preferring P line (S37–S38 generations) were obtained from the Indiana University School of Medicine breeding colonies. The rats were alcohol-naive at the start of the experiments and weighed 275–300 g. Female rats were used because they maintain their weight better than males do within a range necessary for accurate stereotaxic placements of guide cannulae, and previous experience in our laboratories indicates no consistent fluctuations in alcohol drinking behavior with the estrous cycle. Animals were housed individually in a vivarium with a reversed light–dark cycle (lights off at 0900). Six separate groups of animals were used in the microinjection experiments for (a) carbachol into the PPN, (b) scopolamine into the PPN, (c) methylscopolamine into the VTA, (d) carbachol into the VTA, (e) neuroanatomical controls for the PPN experiments and (f) neuroanatomical controls for the VTA experiments.

All rats were stereotaxically implanted under pentobarbital anesthesia (45 mg/kg, IP) with bilateral 26-gauge guide cannulae aimed at the PPN or VTA and, in the case of the neuroanatomical control experiments, aimed at sites 2 mm dorsal and lateral to the target region. The stereotaxic coordinates of Paxinos and Watson (22) were used for implanting cannulae at the target sites. The coordinates (in mm from bregma) were AP −5.3, L ±2.8, V −7.4 (implanted at a 14° angle from the vertical) for the VTA and AP −7.3, L ±3.0, V −6.5 (implanted at a 9° angle from the vertical) for the PPN. The tip of each guide cannula was positioned 1 mm above the target site. A stylet, which protruded 1.0 mm beyond the tip of the guide cannula, was inserted whenever the microinjector was not in place. The 33-gauge microinjector extended 1.0 mm beyond the tip of the guide. Animals were allowed approximately 2 weeks to recover from surgery before initiating the microinjection phase of the experiments.

All animals were implanted bilaterally with guide cannulae. However, initial studies using unilateral microinjection of scopolamine into the PPN have indicated that significant effects on ethanol intake can be observed with this experimental approach. Therefore, all subsequent studies were undertaken with unilateral microinjections. This approach proved to be advantageous because, with the maximum number of microinjections limited to 5–6 per site, each P rat could be used for twice the number of experiments. Systematic comparisons of bilateral with unilateral microinjections were not carried out, and there appeared to be no differences between unilateral injections given on the left side vs. the right side. Although the reason for the effectiveness of unilateral microinjections is unknown, one possibility is that bilateral information from the PPN and VTA may converge and become integrated at other sites (e.g., nucleus accumbens, ventral pallidum, etc.); another possibility is that alteration of inputs from one side is sufficient to change the integrated outflow from downstream sites.

Scopolamine hydrochloride, a nonselective muscarinic antagonist, was purchased from Sigma Chemical Company (St. Louis, MO); carbachol, a cholinergic agonist, (−)-methylscopolamine bromide, a nonselective muscarinic antagonist, and oxotremorine-M, a muscarinic agonist, were purchased from Research Biochemicals International (Natick, MA). Scopolamine was used in initial studies, and methylscopolamine was used in subsequent studies since the methylated compound may have a lower diffusion rate than scopolamine (26). All drugs were dissolved in artificial cerebrospinal fluid (aCSF; composition in mM: NaCl, 120; KCl, 4.75; $CaCl_2$, 2.5; $NaHCO_3$, 25; KH_2PO_4, 1.2; $MgSO_4$, 1.2; pH 7.4) and microinjected by using a Hamilton 10-μl syringe with a Harvard Apparatus 22 pump. The total volume of 0.5 μl was administered over a 30-s period, and the injector was left in place an additional 30 s.

Rats were trained in receiving a daily 2-h scheduled access to a 10% (v/v) ethanol solution and a 0.0125% (g/100 ml) saccharin solution in a two-bottle choice paradigm. Saccharin intake was measured to assess nonselective effects of the pharmacological manipulations. The 2-h access period for each rat occurred at the same time every day, and all access periods occurred between 0900–1200 within the first 4 h after onset of the dark cycle. The intakes of the ethanol and saccharin solutions were recorded at 15, 30, 60 and 120 min of the 2-h access. Water was available during the remaining 22 h, and food was available ad libitum throughout the experiments; the 22-h water and 2-h food intakes were recorded. Food consumption is too small and variable to measure at intervals shorter than the 2-h interval. The rats were weighed at least once a week.

Stable intakes of the ethanol and saccharin solutions were established before microinjections of agents (<15% day-to-day variation over 5 days). Microinjections began with vehicle (aCSF) and continued with the drug doses in randomized order. The microinjections were performed immediately before scheduled access sessions. The doses of each agent tested were: scopolamine (5, 10 or 15 μg/0.5 μl or 15, 30 and 45 nmol/0.5 μl), carbachol (1, 2 or 4 μg/0.5 μl or 5, 10 and 20 nmol/0.5 μl), oxotremorine-M (0.1, 0.3 or 0.5 μg/0.5 μl or 0.3, 1.0 and 1.5 nmol/0.5 μl) and methylscopolamine (1, 5 or 10 μg/0.5 μl or 2.5, 12.5 and 25 nmol/0.5 μl). Each rat received a maximum of six injections per side, and the intakes of ethanol and saccharin were monitored for at least 3 days between injection days. This procedure ensured that the viability of a site was not compromised, no drug carryover effects had occurred and the intakes of ethanol and saccharin had returned to baseline levels before the next microinjection.

To verify the placement of the injector tips in the PPN or VTA, rats were anesthetized, infused with trypan blue (0.5 μl/

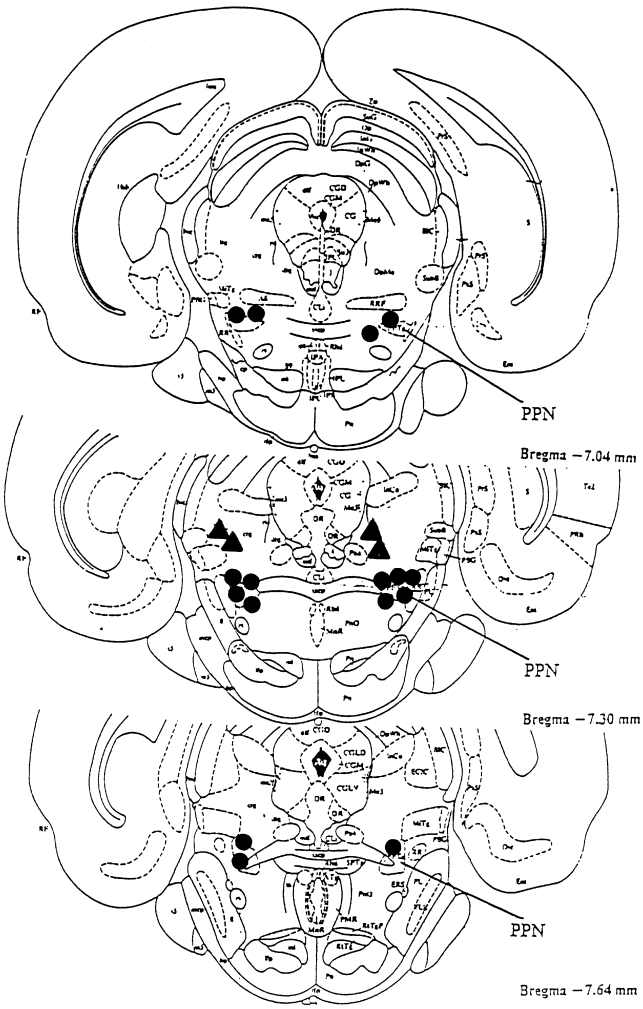

FIG. 1. Location of cannula placement sites for PPN carbachol microinjections. Solid circles indicate cannula placements within the PPN; solid triangles indicate placements outside the PPN. [Modified from Paxinos and Watson (22); reproduced with permission from Academic Press.]

30 s) and decapitated. The brains were removed and frozen. Frozen sections were prepared and the site of injection was assessed. The data for accurate placements were analyzed by a two-way repeated-measures analysis of variance (dose × time). Newman–Keuls post hoc comparisons were performed for each dose and time.

RESULTS

Carbachol Microinjections into the PPN

Stereotaxic placements were within the PPN for 8 of 10 animals, and the other two rats in this group had placements dorsal to the PPN (Fig. 1). Carbachol (1–4 μg or 5–20 nmol) microinjected into the PPN caused a dose-dependent decrease in ethanol intake of P rats [$F(3,21) = 5.65$; $p = 0.005$] (Fig. 2). This effect was most pronounced within the first 30 min; by 2 h, ethanol intake had recovered to near baseline values, except at the highest dose of carbachol. Ethanol intake increased over time, resulting in a main effect for time

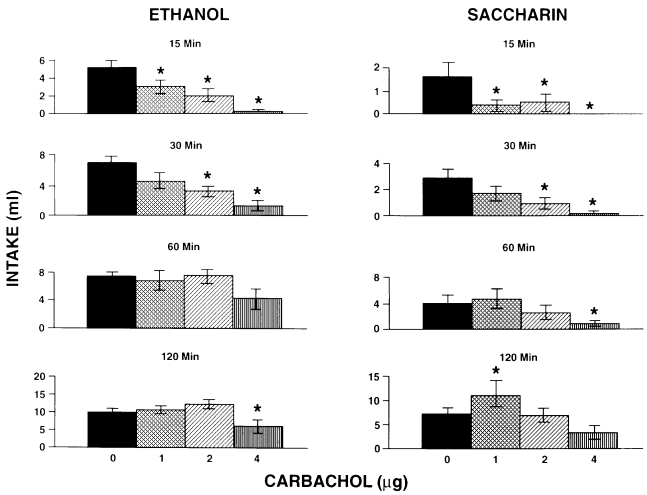

FIG. 2. Effects of aCSF (0) or carbachol (1, 2 or 4 μg; 5–20 nmol) microinjected unilaterally into the PPN on 10% (v/v) ethanol and 0.0125% (g/v) saccharin consumed after 15, 30, 60 and 120 min by P rats ($n = 8$). Control ethanol intake/2 h was 2.3 ± 0.2 g/kg; control saccharin intake/2h was 25 ± 3 ml/kg. Data are the means (± SEM). *$p < 0.05$ vs. vehicle values.

[$F(3,21) = 167.32$; $p < 0.0001$], and higher doses of carbachol tended to act longer, yielding a significant dose × time interaction [$F(9,63) = 3.48$; $p = 0.002$]. Compared with control values, the 1 μg dose produced an approximate 50% decrease in ethanol intake, which was evident only within the first 15 min. The 2 μg dose produced an initial decrease at the 15- and 30-min time points, which was not evident by the end of the session. The 4 μg dose of carbachol completely abolished etha-

TABLE 1

EFFECTS OF CHOLINERGIC AGENTS MICROINJECTED INTO THE PPN OR VTA ON FOOD AND WATER INTAKES

Dose (μg/microinjection)	Food (g/2 hr)	Water (ml/22 hr)
Carbachol/PPN		
0	6.0 ± 0.7	30 ± 2
1	6.7 ± 1.1	27 ± 1
2	6.9 ± 1.0	31 ± 3
4	7.3 ± 1.9	32 ± 2
Scopolamine/PPN		
0	4.8 ± 1.7	33 ± 2
5	4.4 ± 0.6	31 ± 2
10	6.3 ± 1.1	27 ± 1
15	5.3 ± 0.7	28 ± 2
Methylscopolamine/VTA		
0	5.8 ± 1.3	37 ± 2
1	4.8 ± 0.6	34 ± 1
5	5.4 ± 1.1	35 ± 3
10	5.2 ± 0.7	36 ± 3
Carbachol/VTA		
0	4.2 ± 0.3	25 ± 1
1	4.3 ± 0.3	31 ± 1*
2	4.4 ± 0.4	33 ± 2*

Data are the means ± SEM.
*$p < 0.05$ compared with control values.

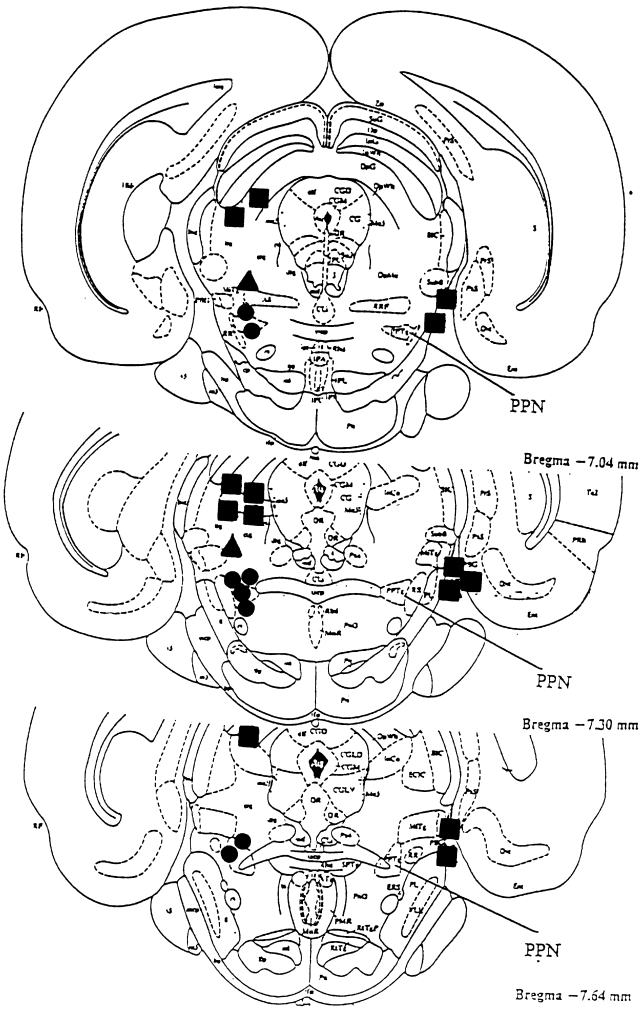

FIG. 3. Location of cannula placement sites for PPN scopolamine microinjections. Solid circles indicate placements within the PPN, and solid triangles indicate placements outside the PPN for the experiment in which scopolamine was microinjected into the PPN; solid squares indicate placements outside the PPN for the neuroanatomical control experiment in which scopolamine was microinjected into sites dorsal and lateral to the PPN. [Modified from Paxinos and Watson (22); reproduced with permission from Academic Press.]

nol intake during the first 15 min, and, by the end of the 2-h session, ethanol intake had recovered to only about 50% of control values.

Saccharin intake was also dose-dependently decreased by carbachol [$F(3,21) = 6.54$; $p = 0.003$]. In addition, there was a main effect of time [$F(3,21) = 17.35$; $p < 0.0001$] and a significant dose × time interaction [$F(9,63) = 4.78$, $p < 0.0001$] (Fig. 2). The highest dose of carbachol reduced saccharin intake by almost 100% within the first 30 min of the scheduled access. At the highest (4 μg) dose, almost complete suppression of saccharin intake persisted for up to 60 min. Effects of the lowest dose wore off within 30 min, and, by the end of the 2-h session, P rats receiving this dose had rebounded with higher saccharin intake.

Carbachol had no main effect on 2-h food intake [$F(3,21) = 0.21$, $p = 0.88$] or 22-h water intake [$F(3,21) = 0.34$, $p = 0.79$] (Table 1). In two rats, microinjection of the 4 μg dose into the

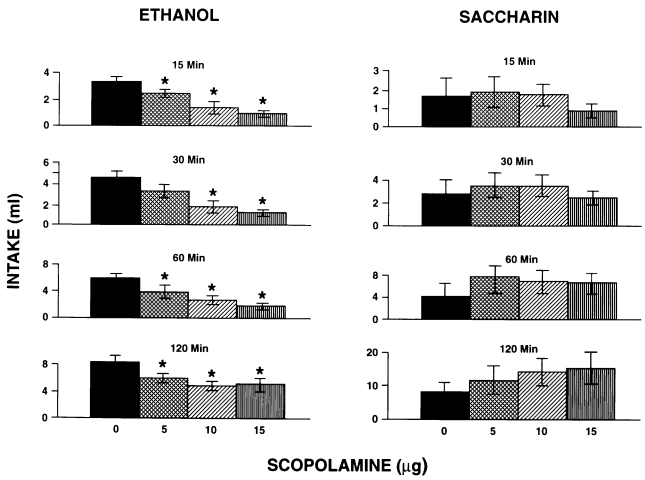

FIG. 4. Effects of aCSF (0) or scopolamine (5, 10 or 15 μg; 15–45 nmol) microinjected unilaterally into the PPN on 10% (v/v) ethanol and 0.0125% (g/v) saccharin consumed after 15, 30, 60 and 120 min by P rats ($n = 8$). Control ethanol intake/2 h was 2.2 ± 0.2 g/kg; control saccharin intake/2 h was 27 ± 8 ml/kg. *$p < 0.05$ vs. vehicle values.

PPN caused brief seizures that dissipated within 5 min; these two animals were returned to the drinking cage only after seizures had subsided. In the two animals with cannula placements dorsal to the PPN (Fig. 1), ethanol and saccharin intakes were not altered by microinjections of carbachol.

Scopolamine Microinjected into the PPN

Eight animals had placements within the PPN and two had placements dorsal to the PPN (Fig. 3). Scopolamine (5–15 μg or 15–45 nmol) microinjected into the PPN produced a dose-related reduction in ethanol drinking within the first 60 min and as much as 75% at the highest dose [$F(3,21) = 16.13$, $p < 0.0001$] (Fig. 4). Ethanol intake did not fully recover by the end of the 2-h limited access period for any of the doses tested. A main effect of time was found [$F(3,21) = 39.85$, $p < 0.0001$] because ethanol intake after the scopolamine microinjections tended to increase over the 2-h session. However, there was not a significant dose × time interaction [$F(9,63) = 1.52$, $p = 0.16$].

Saccharin intake of the P rats within the first 15 min tended to decrease following the 15 μg dose of scopolamine (Fig. 4), but then intake increased approximately twofold by 120 min after injection of the two highest doses (10 and 15 μg), resulting in a main effect of time [$F(3,21) = 8.99$, $p = 0.0005$], dose [$F(3,21) = 2.86$, $p = 0.04$] and a dose × time interaction [$F(9,63) = 4.59$, $p = 0.0001$].

Food consumed during the access period [$F(3,21) = 1.81$, $p = 0.17$] and 22-h water intake [$F(3,21) = 2.27$, $p = 0.11$] were not significantly altered by scopolamine microinjected into the PPN (Table 1). The 10 and 15 μg doses of scopolamine appeared to elicit hyperactivity and increased sniffing and exploration in P rats, which was observed only within the first 5 min following injection. The intakes of ethanol and saccharin by the two animals with injection sites dorsal to the PPN (Fig. 3) were not altered by scopolamine.

Methylscopolamine Microinjected into the VTA

Stereotaxic placements of the cannula were within the VTA for nine rats and dorsal to the VTA in three rats (Fig. 5;

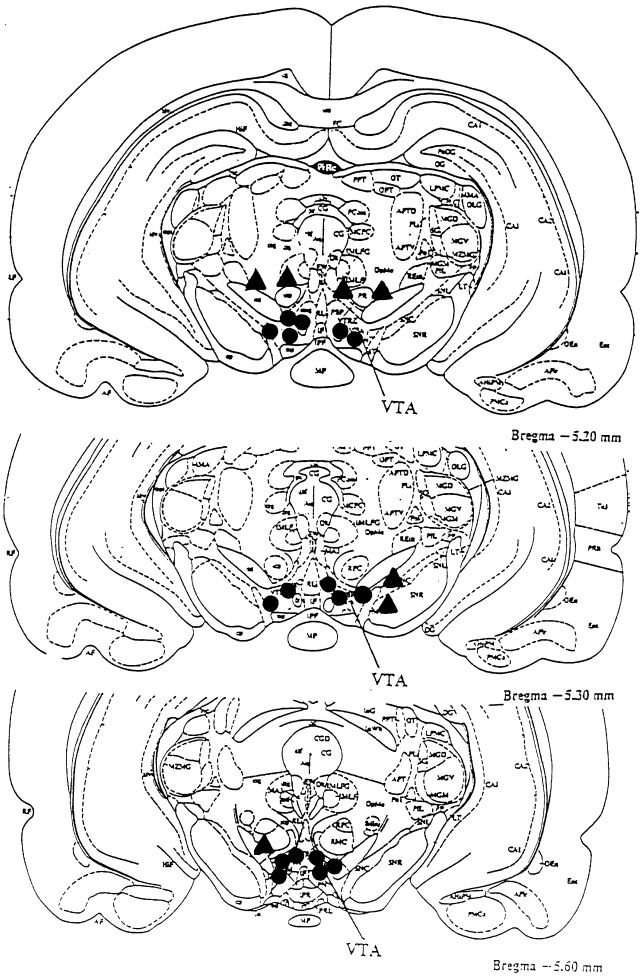

FIG. 5. Location of cannula placement sites for VTA methylscopolamine microinjections (left) and oxotremorine-M (right). Solid circles indicate placements within the VTA, and solid triangles indicate placements outside the VTA. [Modified from Paxinos and Watson (22); reproduced with permission from Academic Press.]

left side of diagram). Methylscopolamine (1–10 μg or 2.5 – 25 nmol) microinjected into the VTA caused a dose-related reduction of ethanol consumption in P rats [$F(3,24) = 17.62, p < 0.0001$] (Fig. 6), which persisted with only a moderate recovery of intake through the end of the 2-h session. A main effect of time was found [$F(3,24) = 46.09, p < 0.0001$], but the dose × time interaction was not reliable [$F(9,72) = 1.63, p = 0.12$].

Saccharin intake was also depressed throughout the 2-h session by the two highest doses of methylscopolamine [$F(3,24) = 4.71, p = 0.01$] (Fig. 6). A partial recovery of intake over the session produced a main effect of time [$F(9,72) = 11.32, p < 0.0001$] and a significant dose × time interaction [$F(9,72) = 4.34, p < 0.0001$].

Food intake during the 2-h access period [$F(3,24) = 0.22, p = 0.88$] and 22-h water consumption [$F(3,24) = 0.25, p = 0.85$] were not altered by injection of methylscopolamine into the VTA (Table 1). In most of the rats tested, the highest dose produced brief ipsilateral turning that dissipated within 5 min. The intakes of ethanol and saccharin by the three animals with placements dorsal to the VTA (Fig. 5) were not altered by methylscopolamine.

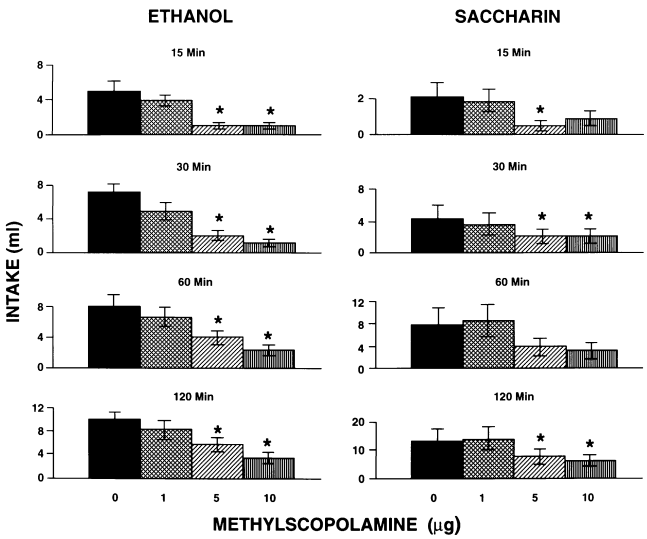

FIG. 6. Effects of aCSF (0) or methylscopolamine (1, 5 or 10 μg; 2.5–25 nmol) microinjected unilaterally into the VTA on 10% (v/v) ethanol and 0.0125% (g/v) saccharin consumed after 15, 30, 60 and 120 min by P rats ($n = 9$). Control ethanol intake/2 h was 2.5 ± 0.3 g/kg; control saccharin/2 h was 36 ± 15 ml/kg. Data are the means (±SEM). *$p < 0.05$ vs. vehicle values.

Carbachol Microinjected into the VTA

Cannula placements were located in the VTA in 9 of 12 animals and dorsal to the VTA in three animals (Fig. 7). Carbachol (1 and 2 μg) microinjected into the VTA produced a dose-related decrease in ethanol intake [$F(2,16) = 4.41, p = 0.03$] (Fig. 8). The 2 μg dose reduced ethanol intake approximately 50% after 60 min. At 15 min, the 1 μg dose reduced ethanol intake by 35%, and the 2 μg dose reduced intake by 80%. By the end of the 2-h session, ethanol drinking had returned to or slightly above baseline values for both doses of carbachol, yielding a main effect of time [$F(3,24) = 50.46, p < 0.0001$] and a significant dose × time interaction [$F(6,48) = 6.78, p < 0.0001$].

Saccharin intake was dose-relatedly increased [$F(2,16) = 6.92, p = 0.007$] following microinjection of carbachol into the VTA (Fig. 8). After 30, 60 and 120 min, the 2 μg dose increased saccharin consumption to 200–270% of control values. This increase in saccharin consumption caused a main effect of time [$F(3,24) = 24.35, p < 0.0001$] and a significant dose × time interaction [$F(6,48) = 2.33, p = 0.046$].

Two-hour food intake was not altered by carbachol [$F(2,14) = 0.19, p = 0.82$] (Table 1). However, 22-h water intake was increased approximately 25% over control values following both doses of carbachol [$F(2,14) = 10.2, p = 0.002$] (Table 1). This difference may have been caused by a slightly lower control value and the low variability among the values.

Oxotremorine-M Microinjected into the VTA

Cannula placements were within the VTA for 8 of 12 rats. Two rats had placements in the substantia nigra, and two others had placements dorsal to the VTA (Fig. 5, right side of diagram). Oxotremorine-M (0.1–0.5 μg or 0.3–1.5 nmol) microinjected into the VTA decreased ethanol consumption by approximately 70% at the highest dose (15-min control and 0.5 μg oxotremorine-M values were 4.3 ± 0.7 ml or 1.1 ± 0.2 g/kg

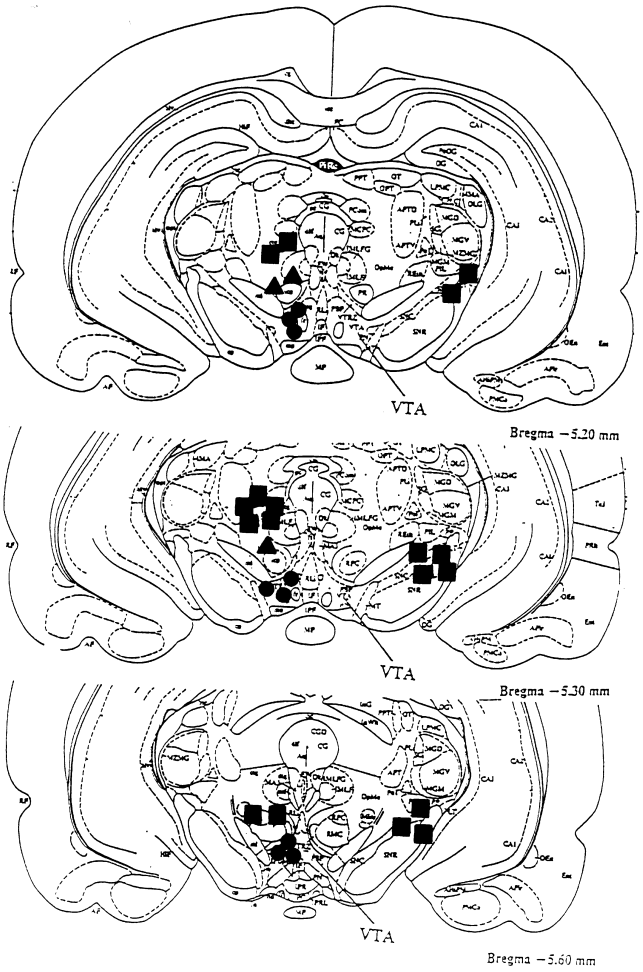

FIG. 7. Location of cannula placement sites for VTA carbachol microinjections. Solid circles indicate placements within the VTA, and solid triangles indicate placements outside the VTA; solid squares indicate placements outside the VTA for the neuroanatomical control experiments in which carbachol was microinjected into sites dorsal and lateral to the VTA. [Modified from Paxinos and Watson (22); reproduced with permission from Academic Press.]

and 1.3 ± 0.3 ml or 0.3 ± 0.1 g/kg, respectively), but the effect was significant only within the first 15 min [$F(3,21) = 4.57$, $p = 0.013$]. After 30 min, control and 0.5 μg oxotremorine-M values were similar [5.5 ± 1.1 ml or 1.4 ± 0.3 g/kg and 4.0 ± 1.5 ml or 1.0 ± 0.4 g/kg, respectively; $F(3,21) = 1.38$, $p = 0.28$]. During the first 15 min, control saccharin intake (3.5 ± 0.9 ml or 12 ± 3 ml/kg) was not altered [$F(3,21) = 0.95$, $p = 0.43$] by the infusion of 0.5 μg oxotremorine-M (2.1 ± 0.9 ml or 7 ± 3 ml/kg) into the VTA. Furthermore, saccharin intake was not altered at other time points (data not shown).

Neuroanatomical Control Experiments

Scopolamine was microinjected into sites where cannulae were aimed dorsal and lateral to the PPN in P rats (Fig. 3). Control values for 2-h ethanol and saccharin intakes were 9.5 ± 1.7 ml (2.4 ± 0.4 g/kg) and 6.0 ± 2.7 ml (20 ± 9 ml/kg), respectively, for the rats with lateral placements and 8.4 ± 0.6 ml (2.1 ± 0.2 g/kg) and 7.7 ± 2.3 ml (26 ± 8 ml/kg), respectively, for rats with the dorsal placements. When the 15 μg dose of

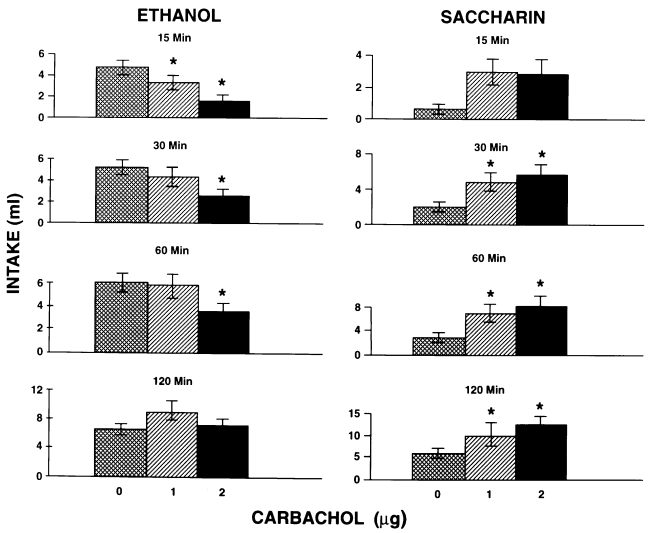

FIG. 8. Effects of aCSF (0) or carbachol (1 or 2 μg; 5 or 10 nmol) microinjected unilaterally into the VTA on 10% (v/v) ethanol and 0.0125% (g/v) saccharin consumed after 15, 30, 60 and 120 min by P rats ($n = 9$). Control ethanol intake/2 h was 2.1± 0.2 g/kg; control saccharin intake/2 h was 23 ± 3 ml/kg. Data are the means (±SEM). *$p < 0.05$ vs. vehicle values.

scopolamine was microinjected into sites dorsal to the PPN, there was no significant effect on ethanol or saccharin intakes at any time point during the 2-h session [$t(6) \leq 2.01$, NS; data not shown]. Two doses of scopolamine (5 and 15 μg) were tested at sites lateral to the PPN and had no effect on ethanol consumption [$F(2,10) = 3.15$, $p = 0.09$] or saccharin consumption [$F(2,10) = 1.68$, $p = 0.23$] in P rats (data not shown). For the control experiments in which carbachol was microinjected into sites dorsal or lateral to the VTA (Fig. 7), the 2 μg dose had no effect on ethanol or saccharin intakes when administered into sites dorsal [$t(8) \leq 0.78$ for ethanol; $t(8) \leq 1.67$ for saccharin] or lateral [$t(6) \leq 1.50$ for ethanol; $t(6) \leq 1.62$ for saccharin] to the VTA (data not shown).

DISCUSSION

Microinjection of scopolamine into the PPN (Fig. 4) and carbachol into the VTA (Fig. 8) of P rats decreased drinking of the 10% (v/v) ethanol solution, with little effect or an increase in saccharin intake (Figs. 4 and 8), suggesting that increasing cholinergic activity of the PPN and VTA may selectively reduce alcohol intake. However, microinjection of carbachol into the PPN and methylscopolamine into the VTA decreased both ethanol (Figs. 2 and 6) and saccharin (Figs. 2 and 6) consumption in P rats, possibly indicating that tonic cholinergic activity of the PPN and VTA may be necessary to maintain general consummatory behaviors. Overall, these results suggest that cholinergic mechanisms within the PPN and VTA are involved in regulating alcohol drinking behavior of the P line of rats.

Enhanced VTA dopaminergic activity may be associated with oral alcohol self-administration but not with oral saccharin self-administration in P rats (31). In the VTA, muscarinic receptors are excitatory (6,16), and administration of carbachol can produce CPP in rats (34). In the present study, activation of muscarinic receptors in the VTA by carbachol may have decreased ethanol intake of P rats (Fig. 8) by mimicking the actions of ethanol on the VTA dopamine system. This in-

terpretation can also be extended to include the effects of microinjecting scopolamine into the PPN (Fig. 4). Because scopolamine inhibits muscarinic receptors in the PPN (36), which may be autoreceptors (17), and the PPN sends projections to the VTA (9,12,20,33), scopolamine may enhance local cholinergic activity within the PPN, which in turn results in stimulation of VTA dopamine neurons. However, because the PPN sends projections to CNS sites other than the VTA (32), the activation of additional pathways may mediate or contribute to the effects of scopolamine.

Concomitant with the reduction of ethanol intake following microinjection of a muscarinic antagonist into the PPN (Fig. 4) or a muscarinic agonist into the VTA (Fig. 8), there was either no alteration or an increase in saccharin intake, and no effect on 2-h food intake (Table 1). These results suggest that the effects of the muscarinic agents on ethanol intake are not likely a result of alterations in general locomotor activity and/or general consummatory behaviors. One possible explanation for the lack of effect of these muscarinic agents to reduce saccharin intake may relate to the finding that oral self-administration of saccharin by P rats does not appear to be associated with increased VTA dopaminergic activity (31). The increased intake of saccharin following injections of cholinergic agonists into the VTA suggests that mechanisms regulating ethanol and saccharin intakes may be different. Saccharin intake may be monitored exclusively through taste factors rather than through postingestional consequences (19), whereas ethanol intake may be regulated mainly by postingestional factors (30). The increased intake of saccharin following cholinergic agonists injection into the VTA may be a compensatory response to maintain total fluid intake.

Microinjection of carbachol into the PPN significantly reduced both ethanol and saccharin intakes (Fig. 2). If carbachol acts at muscarinic autoreceptors within the PPN (17), then the cholinergic outflow should be reduced. One interpretation of this carbachol effect is that decreasing the output of the PPN reduces the reinforcing actions of ethanol and saccharin. Excitotoxic lesions of the PPN may block the development of CPP to morphine and amphetamine (21), reduce CPP to a 0.025% saccharin solution (28) and disrupt the acquisition of responding for conditioned reinforcement stimulated by *d*-amphetamine (11) without altering locomotor activity. Therefore, the results of the present study are consistent with the findings of the lesion studies and suggest that normal functioning of the PPN is required to maintain the rewarding properties of ethanol and saccharin. Alternatively, microinjection of carbachol into the PPN may decrease locomotor activity in rats (5), and this general depressant action may account for the reduction of both ethanol and saccharin intakes observed in the present study (Fig. 2). Food intake (Table 1) may not have been altered by carbachol because the amount consumed was measured only at the end of the 2-h period, when the effects of the agent would have abated.

Injection of a muscarinic antagonist into the VTA also reduced both ethanol and saccharin intakes (Fig. 6). This effect presumably is a result of blocking excitatory cholinergic inputs to the VTA (6,16). These results suggest that active cholinergic inputs from the PPN (9,12,20,33) and/or laterodorsal tegmental nucleus (4) are required to maintain ethanol and saccharin consumption in particular or to maintain consummatory behaviors in general.

The lack of effect for scopolamine on ethanol intake when microinjected into sites dorsal and lateral to the PPN indicates that diffusion to these sites could not account for the effects of scopolamine administered into the PPN. In addition, the lack of effect of carbachol on alcohol drinking when microinjected into sites dorsal and lateral to the VTA indicates that these sites are not responsible for the effects of carbachol locally applied into the VTA on ethanol drinking behavior.

The present results that scopolamine microinjected into the PPN decreased ethanol intake and increased saccharin intake in P rats are consistent with published findings. Scopolamine administered twice daily (0.5 and 2.0 mg/kg, SC) reduced ethanol intake and increased water intake in P rats (23), and atropine, a muscarinic antagonist, administered twice daily (5 mg/kg SC) reduced ethanol consumption and increased saccharin consumption in Sprague-Dawley rats (27). Also, scopolamine administered intracerebroventricularly (ICV; 20–80 μg) decreased ethanol consumption in a dose-dependent manner without altering food or water consumption in P rats (13). The parallel findings of the systemic, ICV and PPN site-specific studies with scopolamine suggest that the peripheral and central administration effect of scopolamine on ethanol and saccharin intakes may be mediated by inhibiting muscarinic receptors in the PPN. This notion is supported further by the results of Yeomans et al. (35) who proposed that PPN autoreceptors may be involved in eliciting the behavioral effects of systemically administered muscarinic antagonists such as scopolamine because carbachol preinjected in the PPN reduced the hypermotility produced by systemic scopolamine. The carbachol-induced inhibition was not seen when the carbachol injection in the PPN followed the systemic scopolamine injection (35), suggesting that carbachol and scopolamine are competing for the same muscarinic receptors in the PPN.

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