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Cholinergic Antagonists in Ventral Tegmentum Elevate Thresholds for Lateral Hypothalamic and Brainstem Self-Stimulation

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KOFMAN, O. AND J. S. YEOMANS. *Cholinergic antagonists in ventral tegmentum elevate thresholds for lateral hypothalamic and brainstem self-stimulation.* PHARMACOL BIOCHEM BEHAV 31(3) 547–559, 1988.—Frequency thresholds for lateral hypothalamic self-stimulation are elevated following microinjections of atropine into ventral tegmentum (73). Many self-stimulation sites in brainstem are situated near cholinergic cell groups and axons, and ventral tegmentum receives cholinergic afferents terminals. To test the hypothesis that ventral tegmental muscarinic receptors are involved in lateral hypothalmic and brainstem self-stimulation, stimulating electrodes were placed in lateral hypothalmus and dorsal tegmentum near the midbrain-pons border, and cannulae were implanted in ventral tegmentum. Microgram injections of muscarinic antagonists, atropine or scopolamine, or a choline uptake blocker, hemicholinium-3, elevated frequency thresholds for both self-stimulation sites in a dose-dependent and time-dependent fashion. In addition, summation and collision between the two self-stimulation sites was tested using paired-pulse methods (53). Summation ranged from 31 to 87% (i.e., 24 to 47% reductions in frequency threshold were observed at long intrapair intervals), but no collision-like effects were observed at short intrapair intervals. The ventral tegmentum is a likely site for the convergence of dorsal tegmental and lateral hypothalamic self-stimulation pathways.

Cholinergic antagonists Ventral tegmentum Lateral hypothalamus Brainstem Self-stimulation

SELF-STIMULATION can be elicited from various midbrain and pontine sites including the ventrolateral central grey (13, 34, 50), superior cerebellar peduncle (13,50), motor nucleus V (60) and regions bordering on locus coeruleus (9, 13, 15, 34, 46). Although self-stimulation in these sites was once attributed to the activation of ascending noradrenergic neurons (25,46), it has since been shown that noradrenergic neurons are not critical. Lesions of the locus coeruleus or knife cuts of the dorsal and ventral noradrenergic bundles did not impair self-stimulation in the trigeminal motor nucleus (60), locus coeruleus or lateral hypothalamus (8, 9, 12).

The proximity of some central grey self-stimulation sites to the raphe nuclei suggested the involvement of serotonin. However the time course of the effect of the serotonin antagonist, parachlorophenylalanine, on the depression of self-stimulation did not coincide with its serotonin-depleting effects (24).

Furthermore, refractory periods for brainstem self-stimulation substrates are, for the most part, shorter than those of noradrenergic or serotonergic neurons. Refractory periods range from 0.4–1.2 msec at dorsal pontine self-stimulation sites (1,72) and from 0.6–2.4 msec in the dorsal and median raphe sites (47). However, the refractory periods for noradrenergic neurons projecting to the forebrain are longer than 2.6 msec (30,58) and those of serotonergic neurons range from 1.2–5 msec (65). The involvement of nonmonoamine neurotransmitters in brainstem self-stimulation has not been investigated, however.

Several studies suggest that cholinergic receptors in hypothalamus and ventral tegmentum are critical for reward. Carbachol was self-administered by rats into the lateral hypothalamus (41) and elicited a conditioned place preference when injected into the ventral tegmentum (73). Microinfusions of acetylcholine into the ventral tegmental area increased bar-pressing rates for lateral hypothalamic self-stimulation, but suppressed rates at high concentrations (45). Metamyzil, purported anticholinergic agent, attenuated medial forebrain bundle self-stimulation in dogs when injected into the posterior hypothalamus (33). Atropine elevated frequency thresholds for lateral hypothalamic self-stimulation when microinjected into the ventral tegmentum (73), while carbachol attenuated the atropine-induced threshold elevations (32). Hence, in the microgram dose range, intracerebral injections of cholinergic agonists appear to be rewarding, while cholinergic antagonists attenuated lateral hypothalamic reward.

In contrast, peripheral injections of muscarinic blockers arecoline (0.1–3 mg/kg) (42,44), pilocarpine (0.5–4.0 mg/kg)


Bregma 10.3 mm
Bregma 8.3 mm
Bregma 9.8 mm
Bregma 7.8 mm
Bregma 7.3 mm

(40), oxotremorine (0.03 mg/kg) (31) and physostigmine (0.05–2.0 mg/kg) (42,44) attenuated self-stimulation rates. The data from the peripheral injections were interpreted as reflecting central muscarinic inhibition of self-stimulation (40,44).

Muscarinic receptors are concentrated near the A10 dopamine cell group (17) on the dorsolateral border between the interpeduncular nucleus and ventral tegmental area in rats (49) and humans (14). This region displays high levels of glucose uptake following medial forebrain bundle self-stimulation (23,70). Low to moderate levels of muscarinic receptors are also found in the ventral tegmental area (37,55) and substantia nigra (37,49). Since cholinergic afferents to ventral tegmentum appear to arise from brainstem cholinergic nuclei as well as forebrain cholinergic nuclei (2, 68, 69), the role of ventral tegmental cholinergic receptors in brainstem self-stimulation was examined.

Self-stimulation sites in the region of the dorsolateral tegmental nucleus, the pedunculopontine nucleus and superior cerebellar peduncle are near cell groups and axons that contain acetylcholinesterase and choline acetyltransferase (Fig. 1). The major cholinergic brainstem nuclei are in the pedunculopontine region, cuneiform nucleus, superior cerebellar peduncle, lateral lemniscus, central tegmental tract, medial longitudinal fasciculus and, perhaps, the parabrachial nucleus (4, 20, 38, 52). These cell groups extend from the caudal part of the substantia nigra to the subcoeruleus region and are collectively referred to as the Ch 5 division (38). Another group of cholinergic cells (Ch 6) is found within the laterodorsal tegmental nucleus (4, 38, 52). Three major branches of acetylcholinesterase-positive axons comprise the cholinergic dorsal tegmental pathway (67): the lateral dorsal pathway projects from the pedunculopontine and laterodorsal nuclei via the lateral tegmentum, below the inferior colliculus, the medial fibers project along the periaqueductal grey, and the intermediate fibers pass in between. A band of fibers ascends from the pedunculopontine nucleus via the retrorubral field and becomes continuous with the ventral tegmental area.

To determine whether ventral tegmental cholinergic receptors are involved in both lateral hypothalamic and brainstem self-stimulation, frequency thresholds for self-stimulation were measured before and after microinjections of atropine, scopolamine, and the choline uptake blocker, hemicholinium-3 in the ventral tegmentum. In addition, the functional connectivity between the two regions was assessed using the paired-pulse collision method of Shizgal *et al.* (53).

METHOD

Subjects and Surgery

Fourteen male Wistar rats were pretreated with 0.04 mg atropine sulfate and anesthetized with 60 mg/kg sodium pentobarbital. Stainless steel electrodes with 200–240 μm diameter hemispherical tips were stereotaxically implanted in the medial forebrain bundle at the level of lateral hypothalamus and in the dorsal tegmentum or dorsal pons. Coordinates for the lateral hypothalamic electrode were 0.3 mm posterior to bregma, 1.7 mm lateral to the midline, and 8.0 mm below dura (incisor bar at +5.0 mm), and for the brainstem electrode were 8.5 mm posterior to bregma, 1.0 mm lateral and 6.8 mm below dura (bregma and lambda on the same horizontal plane). An electrode wrapped around three jeweller's screws in the skull served as a ground.

In addition, guide cannulae were aimed 0.5 mm above ventral tegmental area in all rats using coordinates 3.3 mm posterior to bregma, 0.9 mm lateral and 7.5 mm below dura (incisor bar at +5.0 mm) or 5.0 mm behind bregma, 1.0 mm lateral and 7.7 mm below dura with bregma and lambda on the same plane. Two control rats (51 and 56) had cannulae aimed 1.0–2.0 mm dorsal to the ventral tegmental cannula site.

Apparatus

The constant-current stimulators used delivered 0.1 msec duration rectangular cathodal pulses. The electrodes were electronically grounded between pulses. Current was monitored by the voltage drop across a 100 Ohm resistor on a Tektronix oscilloscope.

The operant chambers (30×28×30 cm) were constructed from Plexiglas. Depression of a lever (5 cm wide protruding 4 cm into the chamber, 8 cm above the floor) triggered the onset of a train of pulses.

Frequency Threshold Measurement

Animals were initially trained to bar press for a train (300 or 500 msec) of pulses at a frequency of 60 Hz. Next, the rats were trained to bar press for one minute of continuous reinforcement, followed by one minute of extinction. The minimum current for which the rats bar pressed at a frequency of 30 Hz was then determined and held constant for frequency threshold tests. The number of bar presses in each one minute trial was measured for a range of frequencies. Each one minute trial was followed by one minute of extinction. Frequencies were adjusted between trials in 10% steps until at least one trial above and one trial below the criterion rate of bar pressing was obtained. The criterion was set at half the maximum rate for each electrode. The frequency threshold was determined by plotting bar-pressing rate vs. frequency, drawing a line between the two frequencies above and below criterion, and determining the frequency from the intersection of the line with the criterion rate (71).

Injection of Cholinergic Antagonists

At least 5 frequency thresholds were tested via each electrode to establish the baseline before injection. Then, atropine sulphate (10, 30 or 60 μg), scopolamine hydrobromide (60, 80 or 250 μg), or hemicholinium-3 (5, 10, 15, or 30 μg) was injected (0.5 μl over a period of 1 min) into ventral tegmentum using a 1 μl Hamilton microsyringe. The injection cannula protruded 0.5 mm below the guide cannula, except where otherwise indicated. All drugs were dissolved

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FIG. 1. Self-stimulation sites in the pons and caudal medulla reported in 12 previous studies (1, 3, 8, 9, 12, 15, 34, 46, 50, 51, 54, 60) are plotted on sections of Paxinos and Watson's (43) atlas of the rat brain. Circles on the right half represent the positive self-stimulation sites. The triangles depict sites from the present study. Circles on the left represent concentrations of cholinergic neurons in motor (large circles) and other brainstem nuclei, adapted from Armstrong *et al.* (4).

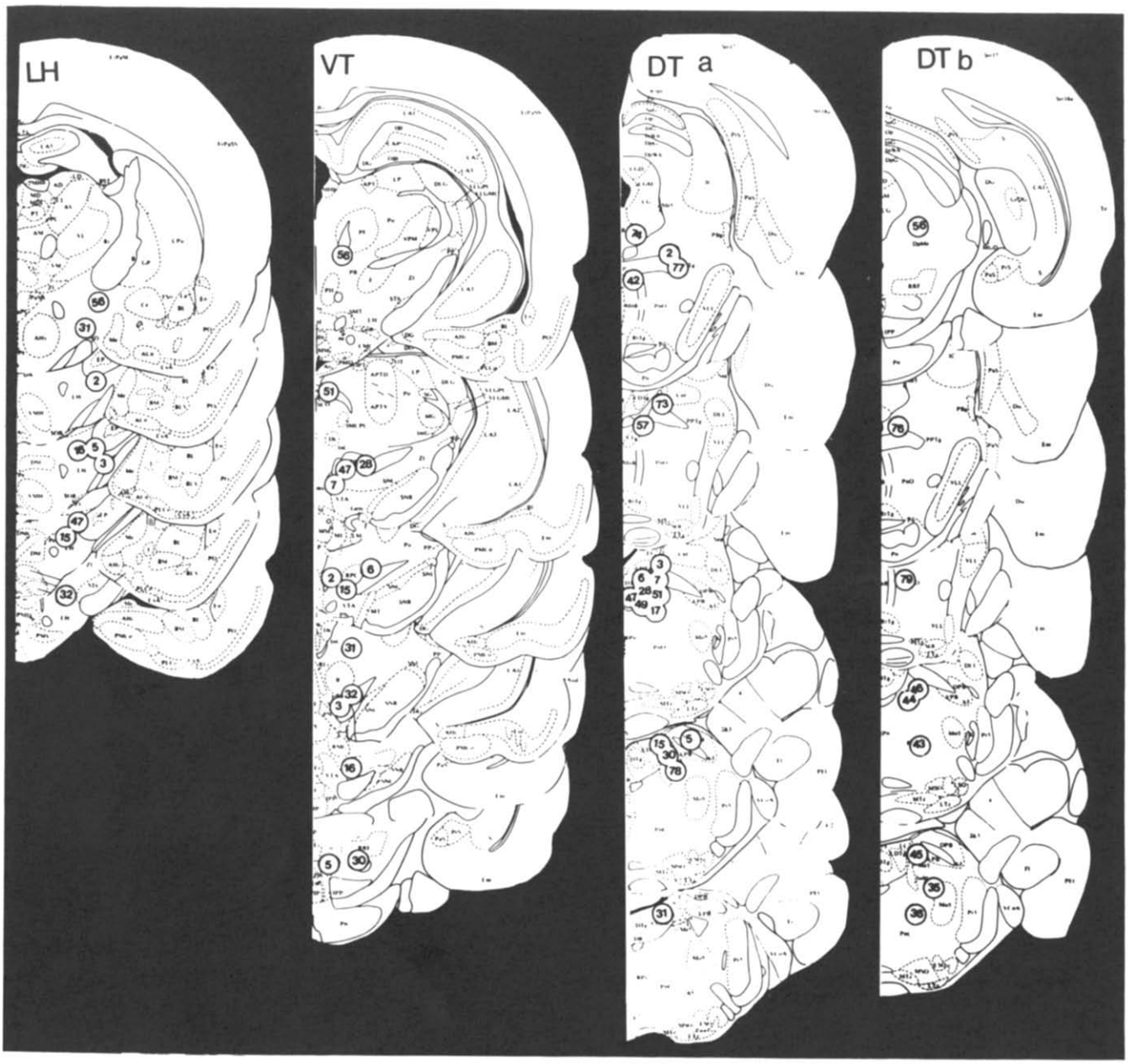

FIG. 2. Sites of stimulation electrodes in lateral hypothalamus (LH) and dorsal tegmentum (DT-A) and injection cannulae in ventral tegmentum (VT) on sections of the Paxinos and Watson (43) atlas. The third panel (DT-A) includes sites that supported self-stimulation of rats not used in the drug studies. The fourth panel (DT-B) shows sites that did not support self-stimulation in the brainstem.

in artifical CSF, which also served as a control injection, except for rat 16 which was injected with Ringer's containing 0.02% ascorbic acid. The pH of the drugs was 7.2–7.4.

In all, nineteen sessions were conducted with atropine sulphate, eight with scopolamine hydrobromide, and eleven with hemicholinium-3 as shown in Table 1. In the earlier rats tested (2, 3, 5–7, 15 and 31), the same doses of cholinergic antagonists were injected in two separate sessions, to determine the reliability of drug effect on sequential tests. Since the results were similar in sequential tests, thresholds obtained with the same dose in a single animal were averaged as described in (72). In the later rats tested, each dose was administered only once, so that a wider range of doses could be tested in each subject.

Brainstem frequency thresholds were tested at five min intervals and lateral hypothalamic thresholds at 20–30 min intervals until thresholds recovered to baseline levels. Drug tests were separated by at least 48 hours.

Bar-Pressing Rates

The mean above-criterion bar pressing rates were com-

pared for the preinjection and postinjection periods for all brainstem electrodes. Rates for lateral hypothalamic self-stimulation are reported only for rats 16 and 32, which had no brainstem electrode, and therefore had many threshold tests. For rats for which both brainstem and lateral hypothalamic electrodes were tested, lateral hypothalamic thresholds were measured only at 20 min intervals in most sessions, and so above-criterion bar-pressing rates are not reported for these lateral hypothalamic electrodes.

RESULTS

Mapping

Positive and negative brainstem self-stimulation sites from 28 animals (including those not used in the drug studies) are mapped on sections of the Paxinos and Watson (43) rat brain atlas in the third and fourth panels of Fig. 2. Since the rats were shaped for up to five days if they showed any signs of approaching the bar, negative sites were defined at those that were clearly aversive or did not elicit any approach response.

Most of the positive sites for self-stimulation were concentrated near the laterodorsal tegmental nucleus, median and dorsal raphe nuclei and near the ventrolateral border of the central grey. Other positive sites were found laterally in the pedunculopontine region. The most caudal sites were in the vicinity of the caudal portion of the laterodorsal tegmental nucleus, adjacent to locus coeruleus. The sites that did not support self-stimulation were near the pontine reticular nuclei and the deep mesencephalic nucleus, although two negative sites (rats 45 and 76) were near sites that generally supported self-stimulation in other subjects. Ambiguous results were obtained from placements just ventral and lateral to central grey, at the level of the superior cerebellar peduncle: some sites were negative, while others required high currents and elicited low bar-pressing rates than the more dorsomedial sites. The distribution of brainstem self-stimulation sites is therefore generally, but not always, consistent with those found in other mapping studies (Fig. 1).

Histology

Histologically verified sites for hypothalamic electrode and cannula placements used in the drug studies are also shown in Fig. 2. Cannula sites for all rats, except the two controls, were located within 1 mm of ventral tegmental area. The guide cannula for control rat 51 was approximately 2.6 mm dorsal to the rostral part of ventral tegmental area with the deepest injection track 1.3 mm below. The guide cannula for rat 56 was approximately 1.8 mm dorsal to ventral tegmental area with the deepest injection site about 1.0 mm below.

The caudal electrodes were spread over a range of about 2 mm extending caudally from the ventrolateral edge of periaqueductal grey to the level of locus coeruleus. The hypothalamic stimulation sites were along the medial forebrain bundle at the level of lateral hypothalamus.

Cholinergic Antagonists

Microinjections of atropine, scopolamine or hemicholinium-3 into ventral tegmentum elevated thresholds for both brainstem and lateral hypothalamic self-stimulation in a dose-dependent fashion. The elevation of frequency thresholds for brainstem self-stimulation was similar to that observed after atropine injections for lateral hypothalamic

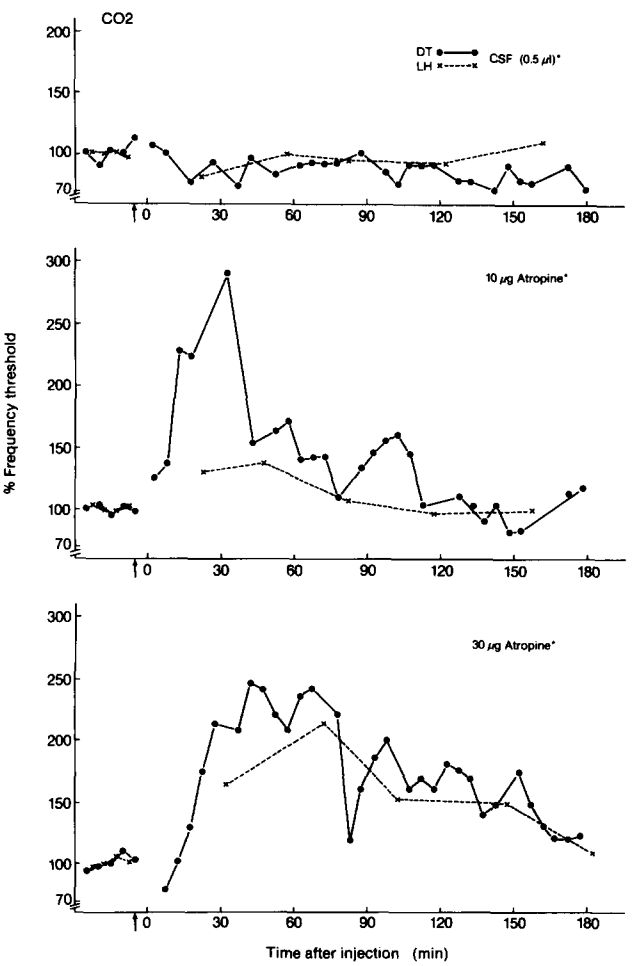

FIG. 3. Frequency threshold changes for lateral hypothalamic and dorsal tegmental self-stimulation following injections of CSF and atropine (10 and 30 µg) in the ventral tegmentum. The abcissa represents the time after injection. The ordinate represents the percent change from the average of 5 preinjection frequency thresholds. The injections were ipsilateral to the site of stimulation.

self-stimulation (73). Sixty µg elicited increases of approximately 100%, while 30 µg increased thresholds approximately 40–50%. Rat 2 showed a 200% frequency threshold elevation after only 10 µg atropine. In other animals, however, this dose resulted only in increased variability in threshold. The atropine effect peaked at 30–50 min. Thresholds returned to baseline gradually, with a half-life for the decay of approximately 45 min (Fig. 3). Scopolamine was less potent and had a slightly shorter time course than atropine (Fig. 4). The time course for drug effects at brainstem self-stimulation sites was more variable than for lateral hypothalamic sites (73).

Occasionally, high doses of atropine (60 µg) elicited ipsiversive turning, leaning and mild ataxia. If turning prevented the animal from responding reliably to priming stimuli within the first half-hour after injection, the session was terminated. Scopolamine, on the other hand, tended to produce transient hyperactivity, including sniffing and grooming behavior, but not turning. The rats generally responded to priming and bar pressed even if they exhibited other stereotyped behaviors.

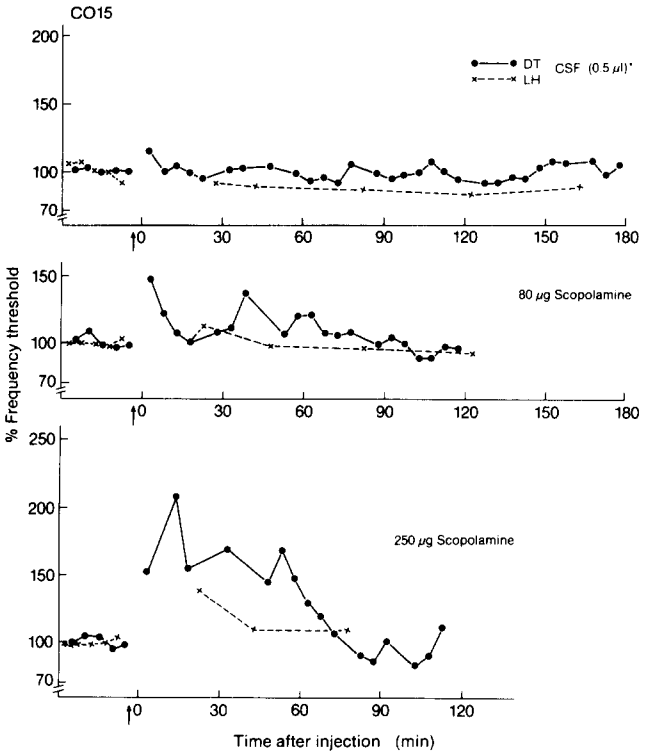

FIG. 4. Frequency threshold changes for lateral hypothalamic and dorsal tegmental self-stimulation following injections of CSF and scopolamine (80 and 250 μg) in the ventral tegmentum. Axes are the same as in Fig. 3.

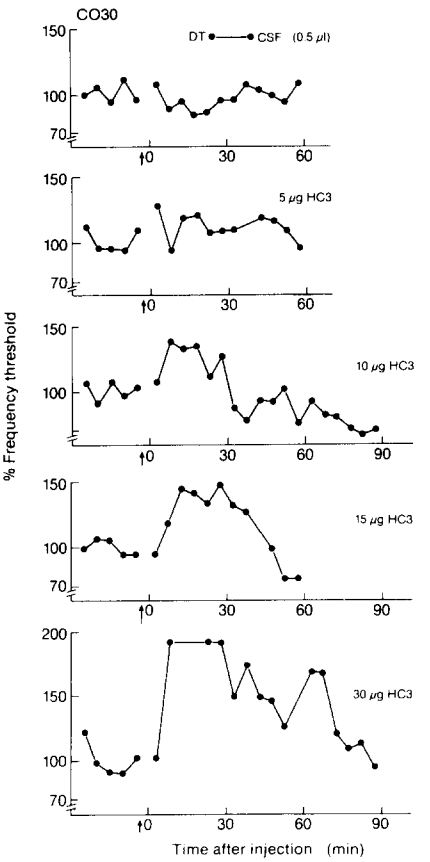

FIG. 5. Frequency threshold changes for dorsal tegmental self-stimulation following injections of CSF and hemicholinium-3 (5, 10, 15 and 30 μg) in the ventral tegmentum. Axes are the same as in Fig. 3.

Injections of the choline uptake blocker, hemicholinium-3, in ventral tegmentum elevated frequency thresholds for brainstem self-stimulation in three rats, and for lateral hypothalamic self-stimulation in three rats. The time course for hemicholinium threshold elevation was similar to that observed after atropine injections, as shown in Fig. 5 for rat 30. Thresholds were increased to more than 150% of baseline between 5 and 50 min after injection of 15 and 30 μg in rats 30 and 31. Five μg injections increased variability slightly in rats 30 and 32, and had no effect on rat 16, while 10 μg increased thresholds to between 140% and 215% of baseline.

The area under the curve for frequency threshold elevations following injections of atropine, scopolamine and hemicholinium-3 in individual subjects is presented in Fig. 6. The vertical axis is minutes of 100% shift from baseline.

Repeated injections had no deleterious effects on self-stimulation frequency thresholds. At lateral hypothalamic sites, thresholds remained fairly constant, whereas at dorsal tegmental sites, thresholds often decreased (Table 1). The latter effect may be due, in part, to increased ability of the rats to cope with stimulus-induced turning from dorsal tegmental electrodes.

Control Injection Sites

Atropine was relatively ineffective in elevating frequency thresholds when injected 2.6 and 1.8 mm dorsal to the ventral tegmental area in control rats 51 and 56 (Fig. 7). These rats were later injected via cannulae that protruded 2 mm and 1 mm below the guide cannula, respectively. The deeper injections of atropine elicited larger frequency threshold elevations.

Above-Criterion Bar-Pressing Rates

The effects of atropine, scopolamine and hemicholinium on mean above-criterion bar-pressing rates were, for the most part, negligible (Fig. 8). In only 6 of 19 sessions were bar-pressing rates following injections of atropine decreased by more than 25% of the baseline rates in the first 30–60 min postinjection. Decreases in bar-pressing rates were seen most often in animals that performed at high rates [e.g., (2, 3, 6)]. However, the frequency threshold elevation had a longer duration than the performance effect.

The same dose of scopolamine (250 μg) elicited a decrement in bar-pressing rates in one session and an increment in the second session for rat 15. Both rats 7 and 15 had erratic bar-pressing patterns and increased rates following injections of CSF and scopolamine. Hemicholinium-3 decreased rates for lateral hypothalamic self-stimulation in rat 16. In all other subjects, rates remained within 25% of baseline throughout the session.

There was no obvious relationship between the drug-induced changes in motor activity and the effects of the drug on bar-pressing rates. For example, hemicholinium-3 caused ataxia, circling and backwards walking in rats 28, 30 and 32, yet bar-pressing rates remained stable. In contrast, rats 31

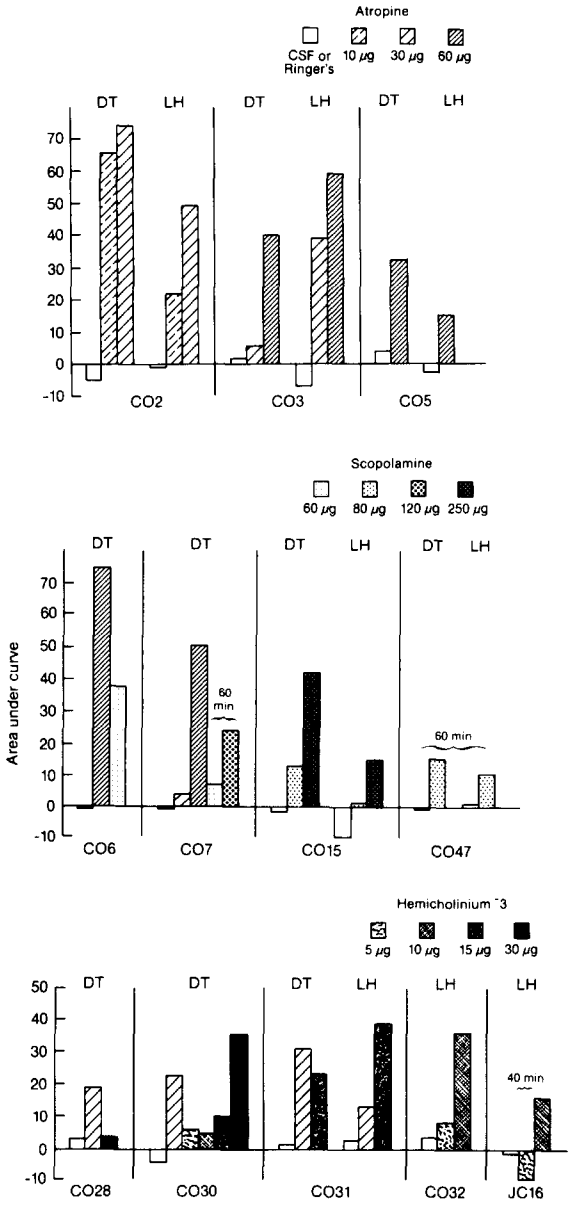

FIG. 6. Summary of the effects of cholinergic antagonists on frequency thresholds. For each session, the area under the curve was measured following injections of CSF, atropine, scopolamine or hemicholinium-3. The ordinate represents the minutes of 100% increase in frequency threshold above preinjection baseline thresholds. The durations tested were 90 min after injection in the upper panels (unless noted by braces) and 60 min after injection in the lowest panels (unless noted by braces).

and 16 had no obvious motor effects with either atropine or hemicholinium-3, but both had decreased rates in the first hour after injection.

EXPERIMENT II: SUMMATION BETWEEN LATERAL HYPOTHALAMIC AND BRAINSTEM SELF-STIMULATION

Frequency threshold elevations for lateral hypothalamic

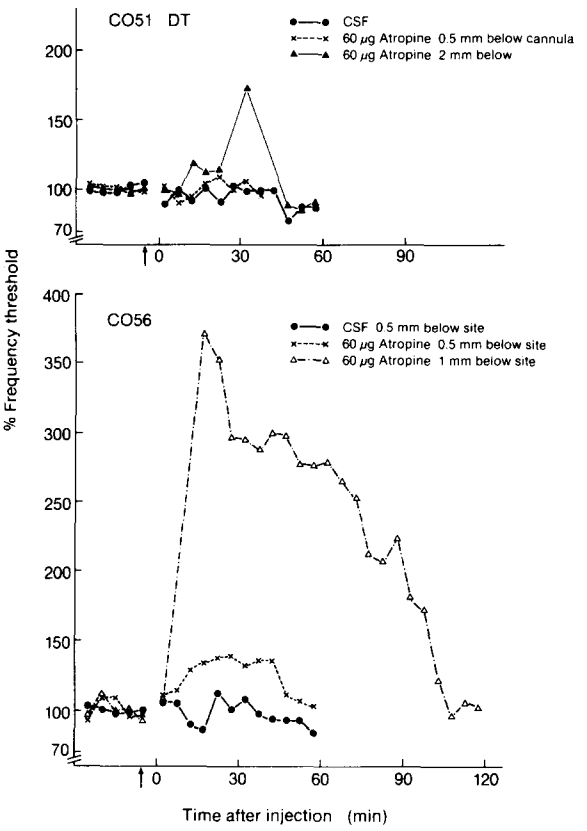

FIG. 7. Frequency threshold changes for lateral hypothalamic self-stimulation following atropine injections via dorsal control cannulae. In both sites, the first injection was much more than 1 mm dorsal to the ventral tegmental area, and the second injection was about 1 mm dorsal to the ventral tegmental area.

and brainstem self-stimulation following microinjections of cholinergic antagonists in ventral tegmentum suggest that there is a reward substrate that is, in part, common to both areas. Lesions of the medial forebrain bundle can disrupt self-stimulation from the brainstem (10) suggesting that ascending substrates may mediate brainstem self-stimulation.

The medial forebrain bundle is composed of approximately 50 ascending and descending fiber systems coursing between the forebrain and brainstem (62), many of which project between the hypothalamic and caudal sites studied here (57, 59, 63, 64). Stimulating electrodes in the lateral hypothalamus and brainstem could, therefore, be activating a common population of axons.

It is not known, however, if any of these long axon groups mediate brain-stimulation reward. In order to determine the connectivity between the reinforcement substrates for medial forebrain bundle and dorsal tegmental self-stimulation, the collision method of Shizgal *et al.* (53) was used. In this test, pulses are delivered in pairs (i.e., one pulse in each pair to each site) at various interpulse intervals and the frequency thresholds measured. If both electrodes directly activate a common axon bundle, then there will be collision between the two action potentials at interpulse intervals shorter than the sum of the refractory period of the axon plus conduction time between the two electrodes. This collision can be meas-

TABLE 1

BASELINE FREQUENCY THRESHOLDS OVER SESSIONS

Animal	Trial	Drug	Current (μA) DT	Current (μA) LH	Threshold (cps) DT	Threshold (cps) LH
2	1	10 μg ATR	200	200	17.5	14
	2	CSF			20.8	17.5
	3	30 μg ATR			19.2	16.5
	4	30 μg ATR			20.8	20
	5	10 μg ATR			17.6	19.3
3	1	CSF	300	800	18.2	27.3
	2	CSF			20.5	24
	3	30 μg ATR			18	22.1
	4	30 μg ATR			18.7	26.2
	5	60 μg ATR			20.2	28.1
	6	60 μg ATR			14.6	23.8
5	1	CSF	150	500	18.3	28
	2	60 μg ATR			18.7	26.5
	3	CSF			23.3	27.5
	4	60 μg ATR			21.3	32.1
6	1	CSF	250		21.24	
	2	60 μg ATR			21.22	
	3	60 μg ATR			21.33	
	4	60 μg SCOP			21.85	
7	1	CSF	400		25.27	
	2	30 μg ATR			18	
	3	CSF			17.6	
	4	30 μg ATR			15.7	
	5	60 μg ATR			14	
	6	60 μg ATR			15.2	
	7	120 μg SCOP	300		14	
	8	60 μg SCOP	300		14.5	
15	1	CSF	500	300	20.8	24.2
	2	80 μg SCOP			17.4	21.6
	3	250 μg SCOP			18.5	18.9
	4	CSF			19.6	20
	5	80 μg SCOP			18.2	20.8
	6	250 μg SCOP			18.2	19.1
28	1	CSF	1000		23.8	
	2	30 μg HC3			23.1	
	3	30 μg ATR			21.4	
30	1	CSF	1500		34.8	
	2	15 μg HC3			34.2	
	3	5 μg HC3			31.5	
	4	10 μg HC3			35.7	
	5	15 μg HC3			34.4	
	6	30 μg ATR			31.3	
	7	30 μg HC3			32.73	
31	1	CSF	800		27.1	
	2	15 μg HC3	500		22.04	
	3	15 μg HC3	500	500	25	45.5
	4	30 μg ATR			25.6	51.3
	5	CSF			25.2	49.1
47	1	CSF	400	800	27.3	41.5
	2	80 μg SCOP			22.6	37.4

(CONTINUED)

TABLE 1
(CONTINUED)

Animal	Trial	Drug	Current (μA) DT	Current (μA) LH	Threshold (cps) DT	Threshold (cps) LH
JC 16	1	Ringer's		800		26.3
	2	10 μg HC3				35.3
	3	5 μg HC3				41.7
32	1	CSF		1000		23.7
	2	10 μg HC3				27.4
	3	5 μg HC3				25.4

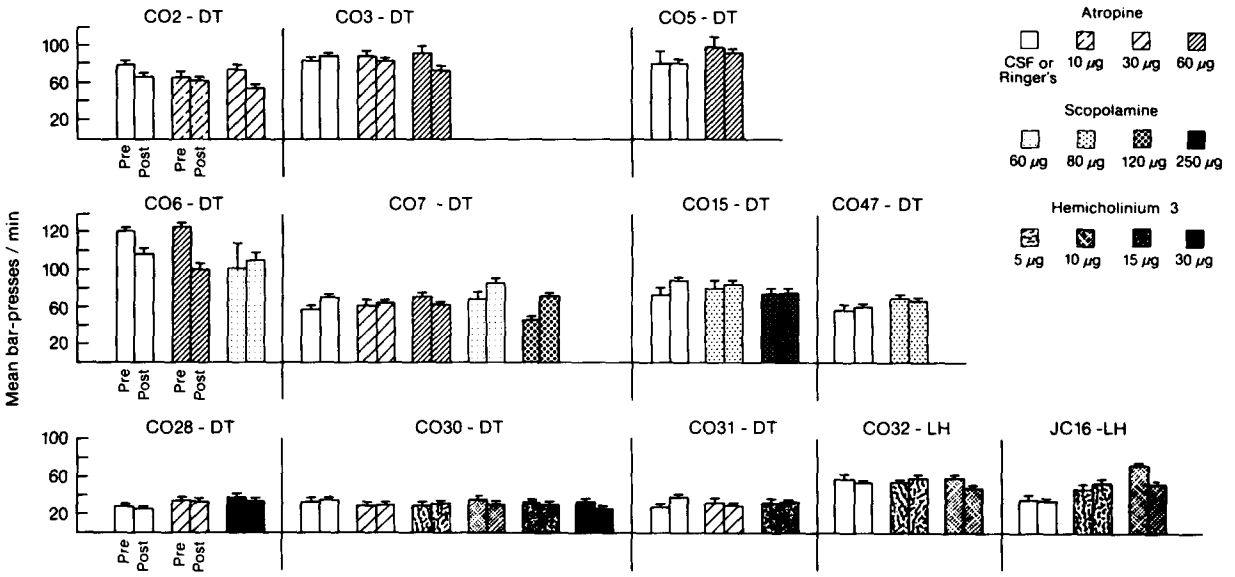

FIG. 8. Mean above-criterion bar-pressing rates for the preinjection and postinjection periods following injections of CSF or anticholinergic drugs in ventral tegmentum. The vertical lines represented standard errors of the mean. The absence of a vertical line indicates that the standard error was too small to be visible on this graph.

ured by a sharp increase in frequency thresholds at interpulse intervals slightly longer than the refractory periods, which are in the 0.4 to 2 msec range (71).

If two pathways are not connected, but converge on a final common pathway, then the frequency thresholds will be similar at all interpulse intervals, but will be less than the frequency thresholds when single pulses are delivered to each site individually. This effect is called summation without collision.

METHOD

Summation was tested on five rats from the previous experiment that had similar bar-pressing rates for both the lateral hypothalamic and brainstem electrodes. The summation experiment was run before any drugs had been injected. The subjects all had cannulae implanted in the ventral tegmentum that were later used in drug studies.

To test the summation between sites, paired pulses were presented, with conditioning (C) pulses delivered to one site and testing (T) pulses to the other site, at long (15–30 msec) intrapair (C-T) intervals. Currents were adjusted so that the frequency thresholds for each site using single pulses were matched. Summation was tested by comparing frequency thresholds with single pulses from each electrode to those obtained under paired pulse conditions at long C-T intervals. To calculate the percent summation, the mean frequency threshold obtained under paired pulse conditions (FT [C-T]) was divided by the mean threshold in the single pulse conditions and 1 subtracted (53):

$$E = [FT\ (C-T)/FT\ (C-C_{LH}) + FT\ (C-C_{DT})/2] - 1$$

where FT (C-C_{LH}) is the average frequency threshold for the single pulse condition on the lateral hypothalamic electrode and FT (C-C_{DT}) is the single pulse frequency threshold on the dorsal tegmental electrode.

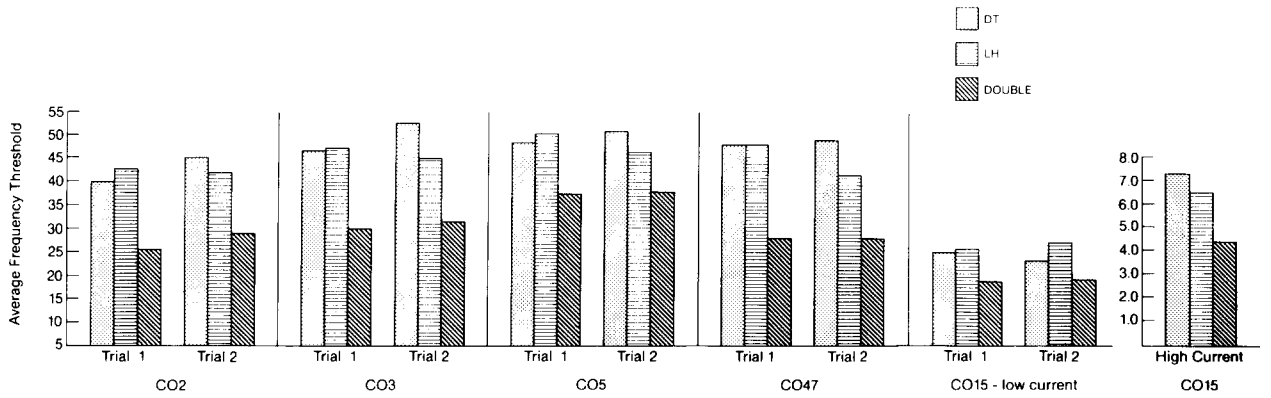

FIG. 9. Frequency thresholds for single-pulse stimulation at lateral hypothalamic (LH) and dorsal tegmental (DT) sites compared to paired-pulse stimulation (DOUBLE) in five rats. In the paired-pulse stimulation, C pulses were delivered via one electrode and T pulses delivered via the second electrode, and the C-T (intrapair) interval was half of the C-C (interpair) interval.

Subsequently, C-T intervals of 0.2 and 0.4 msec were tested to determine if there was collision between the two stimulation sites (53). Seven to nine thresholds were tested in each single-pulse and paired-pulse condition. Two sessions were run per animal.

Rat 15 was tested twice, once using high currents on both electrodes (800 μA for the lateral hypothalamus and 1000 μA for the brainstem at a train duration of 2 sec), and once using low currents (250 μA and 500 μA, respectively, at a train duration of 300 msec).

RESULTS

Frequency thresholds for paired pulse stimulation of the brainstem and lateral hypothalamus at long C-T intervals were 24–47% less than frequency thresholds for each electrode alone (Fig. 9). This suggests that there was good summation between hypothalamic and brainstem sites. Similar results were obtained whether the C pulses were delivered via the rostral or caudal electrode. The lowest level of summation (24% decrease in threshold) was observed in rat 5, whose caudal electrode was located near the parabrachial nucleus.

Frequency thresholds were decreased to the same extent at C-T intervals of 0.2 and 0.4 msec as at long C-T intervals. This suggests that there was no collision between the action potentials initiated at the two stimulation sites (53), but that the reward signals from the two regions are integrated at a common output.

DISCUSSION

Atropine, scopolamine and hemicholinium-3 injected into the ventral tegmentum elevated frequency thresholds for lateral hypothalamic and brainstem self-stimulation, without reliably altering peak bar-pressing rates. These data suggest that ventral tegmental muscarinic receptors are critical for dorsal tegmental self-stimulation as well as for lateral hypothalamic self-stimulation (73).

Measurement Methods

Previous experiments on the effects of cholinergic agents on self-stimulation only recorded changes in bar-pressing rates, which is now recognized to be an inadequate measure of reward (35). Gallistel and colleagues (19,24) have argued that entire rate-intensity functions should be obtained so that the effects of a drug on the locus of rise of these functions and on peak performance can be distinguished.

Peak performance (measured by above-criterion bar-pressing rates) was altered very little by the cholinergic drugs tested here, however. Even when performance effects were observed, they did not last as long as the frequency threshold changes. Therefore, we decided to test thresholds more often than would be possible with the locus-of-rise method, so that the time course of drug action could be measured.

The inconsistent effects on rates are similar to those reported in earlier experiments following systemic injections of muscarinic antagonists. Nonsignificant decrements in bar-pressing rates (40), biphasic stimulation and depressant effects (39,44) and increases in rates (31) have all been reported following peripheral scopolamine injections. Scopolamine increased the bar-pressing rates during the extinction, but not the reward phase of another study (66). Atropine had no effect on bar-pressing rates in some cases (40,61), elicited a response decrement in some subjects and an increment in others (40).

Specificity

Central injections into the hypothalamus or ventral tegmentum appear to be required to obtain these effects. This conclusion is supported by our finding that dorsal injections are ineffective. A problem with intracranial injections, however, is that the tissue near the injection site receives a much greater dose than tissue farther from the injection site. This raises the question of the specificity of drug effects, especially with injections of microgram doses of atropine or hemicholinium-3, which can have local anesthetic effects (16, 18, 22).

In further studies, we have found that the magnitude and duration of frequency threshold elevations following procaine injections were less than those following atropine injections (Kofman, Yeomans and McGlynn, in preparation). Also, scopolamine does not appear to have the local anesthetic effects of atropine and hemicholinium-3. Furthermore, pretreatment with carbachol (1–3 μg) attenuated the atropine effect (32). Therefore, although part of the effects observed here could be due to the local anesthetic

properties of atropine and hemicholinium, the primary effects appear to be anticholinergic.

Relationship Between Hypothalamic and Dorsal Tegmental Self-Stimulation

The ability of cholinergic antagonists in ventral tegmentum to elevate frequency thresholds for both brainstem and lateral hypothalamic self-stimulation suggests that the reward substrates from both these regions may have a common output. This was supported by the summation of the reward signal from the two regions. The failure to obtain collision may be due to the lower currents used here (except for rat 15) than in medial forebrain bundle collision studies. Bielajew and Shizgal (6) found collision only when currents above 500 μA were used. High currents were not used for all subjects in this study since the high currents exacerbated motor effects or impaired frequency following on the brainstem electrodes [see also (1,47)]. Nevertheless, two previous studies have also reported that concurrent stimulation of lateral hypothalamus and ventrolateral central grey (5) or locus coeruleus (7) did not produce collision.

It is not clear if cholinergic axons form part of the directly stimulated substrate for either lateral hypothalamic or brainstem self-stimulation. The ventral tegmental area receives distinct and dense cholinergic inputs from the diagonal band (2) and additional cholinergic projections from habenula, preoptic area, substantia innominata and the bed nucleus of stria terminalis (2,68). In addition, atropine blocked short refractory period axons mediating lateral hypothalamic stimulation (28). However, Grove, Haber, Domesick and Nauta (29) found no cholinergic projections from ventral pallidum to the ventral tegmental area. Moreover, it is not known if cholinergic axons descend from basal forebrain to ventral tegmentum via the medial forebrain bundle, or whether all the cholinergic afferents descend via the habenula and fasciculus retroflexus (11, 20, 26, 52).

The location of brainstem self-stimulation sites is consistent with the suggestion that ascending cholinergic efferents from the Ch 5 and Ch 6 cell groups, which course through the pedunculopotine region adjacent to superior cerebellar peduncle, ventrolateral central grey and raphe nuclei (67) correspond to the more sensitive self-stimulation sites. The relevant pathways for brainstem stimulation reward may be better delineated by systematic studies measuring current-distance relations and collision between sites (21,53).

Although they may not be directly activated by self-stimulation electrodes, cholinergic afferents to ventral tegmentum may be trans-synaptically activated by collateral axons or interneurons, thereby forming a critical link in the reward pathways of lateral hypothalamic and brainstem self-stimulation.

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