Pharmacology Biochemistry & Behavior, Vol. 7, pp. 173–176.

BRIEF COMMUNICATION

Self-stimulation of the Subfornical Organ and Lateral Hypothalamus: Differential Effects of Atropine and Methysergide[1]

A. ROBERTSON, J. KUCHARCZYK[2] AND G. J. MOGENSON

Departments of Physiology and Psychology, University of Western Ontario, London, Canada, N6A 5C1

(Received 16 December 1976)

ROBERTSON, A., J. KUCHARCZYK AND G. J. MOGENSON. *Self-stimulation of the subfornical organ and lateral hypothalamus: differential effects of atropine and methysergide.* PHARMAC. BIOCHEM. BEHAV. 7(2) 173–176, 1977. – The effects of cholinergic blockade of neurons by atropine or serotonergic blockade by methysergide was investigated in rats responding for brain-stimulation reward. Bipolar stimulating electrodes were placed either in the subfornical organ (SFO) or the lateral hypothalamus (LH). Atropine sulphate and methysergide significantly suppressed self-stimulation of the SFO but not of the LH, suggesting that cholinergic and serotonergic neurons are involved in brain-stimulation reward associated with this site.

Self-stimulation SFO Atropine Methysergide Amphetamine

THE SUBFORNICAL organ (SFO), which has gained prominence as the possible site of the dipsogenic effects of angiotensin II [4,14], has been shown recently to subserve brain stimulation reward [11]. There is evidence that this structure contains dopamine or noradrenaline [3, 6, 12], both hypothesized to mediate self-stimulation [7,10]. However the SFO may contain acetylcholine [1, 3, 5] and serotonin [6]. The possibility that these neurotransmitters play a role in self-stimulation of the SFO was investigated by administering atropine, a cholinergic antagonist, and methysergide, a serotonergic antagonist, to rats self-stimulating through SFO electrodes. For comparison, the effects of these drugs on self-stimulation of the lateral hypothalamus (LH) was also examined.

METHOD

Male albino rats weighing 250–300 g at the time of surgery were used in the study. The animals were individually housed in wire mesh cages in a temperature-controlled room with lights on from 7:00 a.m. to 9:00 p.m. and with tap water and Purina rat chow available ad lib. Rats were anaesthetized with sodium pentobarbital (40–50 mg/kg IP) and were implanted under stereotaxic control with bipolar electrodes (Plastic Products Co., Roanoke, V), 127 μ in diameter and insulated except for the cross-sectional area at the tips. With the incisor bar 5.0 mm above the interaural line, electrodes were implanted into the subfornical organ (12° from the vertical midline, 0.0 mm anterior to bregma, 1.0 mm lateral, and 4.4–4.5 mm ventral to dura) and in the medial part of the lateral hypothalamus.

Following a one-week recovery period, the animals were tested for self-stimulation. Testing took place in a Plexiglas box (30 × 16 × 30 cm) with a lever at one end which could be depressed with a force of 16 g to deliver a 0.20 sec train of electrical stimulation. The stimulating waveform provided by a Grass S44 stimulator consisted of monophasic rectangular pulses of 0.20 msec duration presented at 80 Hz through a Grass stimulus isolation unit. Current was monitored continuously on an oscilloscope.

For animals in which self-stimulation occurred, testing was continued daily in one-half hr sessions and current was kept constant at suprathreshold levels which generated approximately equal response rates between groups of rats. Drugs were administered only after response rates were stable (i.e., a change from one day to the next of less than

[1] This research was supported by grants from the Medical Research Council of Canada and the National Research Council of Canada to Dr. G. J. Mogenson. The authors acknowledge the technical assistance of Becky Woodside in preparing the illustrations and Blanche Box for help with the manuscript. Dr. Maurice Bourgouin of Sandoz Pharmaceuticals, Dorval, Quebec kindly supplied the methysergide used in this experiment.

[2] Present address: The Physiological Laboratory, University of Cambridge, Cambridge, England, CB2 3EG.

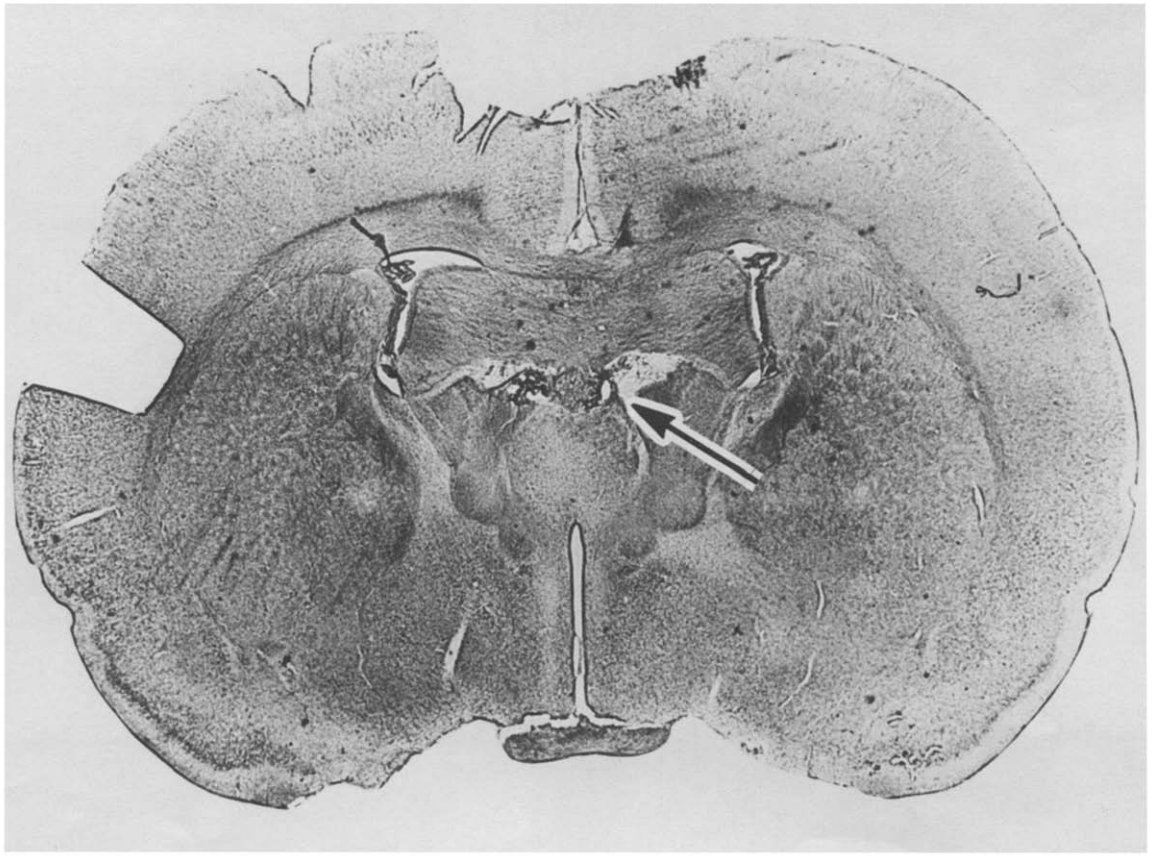

FIG. 1. Coronal section of a rat brain showing a representative electrode placement in the subfornical organ. The tip of the electrode, indicated by the arrow, lies in the lateral part of the subfornical organ.

10%) over a minimum of three daily consecutive tests. A minimum of three days was allowed to elapse between drug administration and no animals received more than three different drug treatments.

Atropine sulphate and atropine methyl nitrate were dissolved in 0.9% isotonic saline and were administered (10, 15, and 20 mg/kg, IP) 20 min before testing. Animals with electrodes in SFO received 10, 15 and 20 mg/kg of atropine sulphate or atropine methyl nitrate; animals with electrodes in LH received 15 and 20 mg/kg of atropine sulphate or atropine methyl nitrate. Methysergide maleate, dissolved to a concentration of 12.5 mg/ml in 0.9% saline and 0.1 N HCl and adjusted with NaOH to a pH of 7.0, was administered in a dose of 25 mg/kg body weight, IP, 20 min before testing. Additionally, three rats with SFO electrodes were administered d-amphetamine and l-amphetamine (1 mg/kg, IP), 30 min before testing.

Upon completion of the behavioral tests, rats were sacrificed by an overdose of sodium pentobarbital. Brain sections of 50 μ thickness were cut using a freezing microtome and were stained with thionin and examined under a microscope. Only those rats with confirmed electrode placements in the SFO and LH were included in the results. A representative electrode placement in the SFO is shown in Fig. 1.

RESULTS AND DISCUSSION

Rates of self-stimulation after various drug treatments were calculated as percentages of the average response rates for the two preceding test sessions. The results are presented in Fig. 2 and Table 1. Atropine sulphate (10 mg/kg) administered to rats with SFO electrodes did not produce a significant suppression of responding compared to the effect of atropine methyl nitrate ($p<0.05$). When given 15 mg/kg atropine sulphate, however, rats with SFO electrodes showed a significant reduction of response rates (to 53% of predrug levels) in comparison to their rates when administered atropine methyl nitrate ($p<0.05$) or in comparison to rats with LH electrodes given atropine sulphate ($p<0.01$). When administered 20 mg/kg atropine sulphate, SFO rats responded at 47% of preinjection levels, significantly lower than their rates following atropine methyl nitrate ($p<0.02$) and lower than response rates of rats with LH electrodes administered 20 mg/kg of atropine sulphate ($p<0.001$).

The administration of 25 mg/kg of methysergide did not reduce self-stimulation of the LH but did, in comparison, significantly reduce self-stimulation of the SFO ($p<0.005$).

The attenuation of self-stimulation from the SFO when a cholinergic antagonist (atropine) or a serotonergic antagonist (methysergide) were administered suggests that both cholinergic and serotonergic neurons are involved in self-stimulation of this area. A nonspecific effect (general behavioral debilitation) can be ruled out since self-stimulation of the LH was not similarly affected. It has previously been reported that atropine sulphate [8] and methysergide [13] have no effect on self-stimulation of the LH. Therefore self-stimulation of the SFO can be differen-

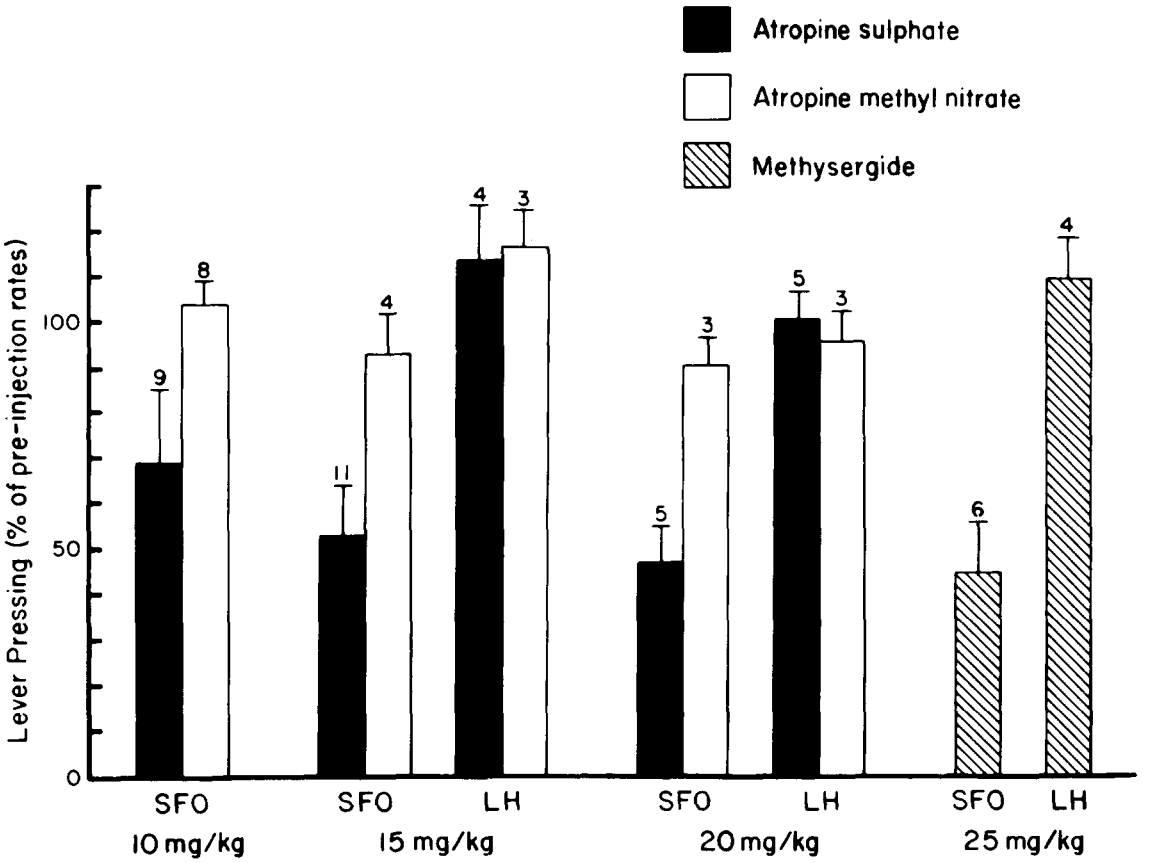

FIG. 2. Response rates for electrical stimulation of the subfornical organ (SFO) or lateral hypothalamus (LH) upon administration of atropine sulphate or atropine methyl nitrate (10, 15 or 20 mg/kg IP, 20 min before testing) or methysergide (25 mg/kg IP, 20 min before testing). Response rates are expressed as percentages of preinjection rates. Vertical bars represent standard errors of the mean. The number of rats in each condition is indicated above each bar.

TABLE 1

THE EFFECT OF d– AND l– AMPHETAMINE* ON SELF-STIMULATION OF THE SFO†

Rat	d	l	Difference
38	347	172	175
42	325	154	171
43	265	128	137
		Mean difference	161

* 1 mg/kg, IP.
† Results expressed as percentage of preinjection response rates.

tiated from self-stimulation of the LH by the responses of animals to these drugs.

Rates of self-stimulation of the SFO were increased following the administration of both isomers of amphetamine, suggesting a possible role for catecholaminergic neurons [10]. The greater facilitation of self-stimulation following d-amphetamine than following l-amphetamine may indicate that noradrenergic neurons might be involved in self-stimulation of the SFO [2, 9, 10]. On the other hand, since self-stimulation of the SFO is suppressed by spiroperidol, a dopamine antagonist (unpublished observations), dopaminergic neurons might also be involved. Because of a recent report that the SFO contains both dopamine and noradrenaline [12] it is possible that activation of catecholaminergic neurons contribute to self-stimulation of the SFO. Alternatively, the attenuation of self-stimulation by spiroperidol might be the result of motoric or other nonspecific behavioral effects.

REFERENCES

1. Akert, K. The mammalian subfornical organ. *J. Neuroviscer. Relat. Suppl.* **IX**: 78–93, 1969.
2. Herberg, L. J., D. N. Stephens and K. B. J. Franklin. Catecholamines and self-stimulation: Evidence suggesting a reinforcing role for noradrenaline and a motivating role for dopamine. *Pharmac. Biochem. Behav.* **4**: 575–582, 1976.
3. Jacobowitz, D. M. and M. Palkovits. Topographic atlas of catecholamines and acetylcholinesterase-containing neurons in the rat brain I. Forebrain (telencephalon, diencephalon). *J. comp. Neurol.* **157**: 13–28, 1974.

4. Kucharczyk, J., S. Y. Assaf and G. J. Mogenson. Differential effects of brain lesions on thirst induced by the administration of angiotensin II to the preoptic region, subfornical organ and anterior third ventricle. *Brain Res.* **108:** 327–337, 1976.
5. Lewis, P. R. and C. C. D. Shute. The cholinergic limbic system: Projections to hippocampal formation, medial cortex, nuclei of the ascending reticular system, and the subfornical organ and supraoptic crest. *Brain* **90:** 521–540, 1967.
6. Lichtensteiger, W. Monoamines in the subfornical organ. *Brain Res.* **4:** 52–59, 1967.
7. Mogenson, G. J. and A. G. Phillips. Motivation: A psychological construct in search of a physiological substrate. *Prog. Psychobiol. Physiol. Psychol.* **6:** 189–243, 1975.
8. Newman, L. M. Effects of cholinergic agonists and antagonists on self-stimulation behavior in the rat. *J. comp. physiol. Psychol.* **79:** 394–413, 1972.
9. Phillips, A. G., S. M. Brooke and H. C. Fibiger. Effects of amphetamine isomers and neuroleptics on self-stimulation from the nucleus accumbens and dorsal noradrenergic bundle. *Brain Res.* **85:** 13–22, 1975.
10. Phillips, A. G. and H. C. Fibiger. Dopaminegic and noradrenergic substrates of positive reinforcement: Differential effects of *d*- and *l*-amphetamine. *Science* **179:** 575–577, 1973.
11. Robertson, A., J. Kucharczyk and G. J. Mogenson. Subfornical organ: A site of brain self-stimulation. *Brain Res.* **114:** 511–516, 1976.
12. Saavedra, J. M., M. J. Brownstein, J. S. Kizer and M. Palkovits. Biogenic amines and related enzymes in the circumventricular organs of the rat. *Br. Res.* **107:** 412–417, 1976.
13. Silveira Filho, N. G. and F. G. Graeff. Effect of tryptamine antagonists on self-stimulation. Interaction with amphetamine. *Psychopharmacology* **52:** 87–92, 1977.
14. Simpson, J. B. and A. Routtenberg. Subfornical organ: Site of drinking elicitation by angiotensin II. *Science* **181:** 1172–1174, 1973.