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Reduction of the Rewarding Effect of Brain Stimulation by a Blockade of Dopamine D1 Receptor With SCH 23390[1]

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NAKAJIMA, S AND G M McKENZIE *Reduction of the rewarding effect of brain stimulation by a blockade of dopamine D1 receptor with SCH 23390* PHARMACOL BIOCHEM BEHAV 24(4) 919–923, 1986.—The subtype of dopamine receptor related to the rewarding effect of brain stimulation was determined in 17 rats The animals were trained to contact a dry spout to receive stimulation through electrodes implanted into the lateral hypothalamic area, ventral tegmental area, or dorsal raphe nucleus The dopamine D1 blocking agent SCH 23390, 0 08 mg/kg IP, completely suppressed responding The D2 receptor blocker sulpiride, 50 mg/kg IP, or the serotonin receptor blocker metergoline, 5 mg/kg IP, did not suppress responding The ED50 for SCH 23390 was 0 022 mg/kg IP In a runway, rats were trained to run for rewarding goal stimulation consisting of a train of pulses delivered to the lateral hypothalamus After injection of SCH 23390, 0 01 mg/kg IP, animals showed significantly slower running speed, but their speed returned to normal if the number of pulses in the goal stimulation was increased 2 6 times These results indicate that blockade of D1 receptors, but not D2 receptors, reduces the rewarding effect of brain stimulation

Brain stimulation reward Dopamine D1 receptor D2 receptor Metergoline SCH 23390 Self-stimulation Sulpiride

A wide variety of neuroleptic drugs interfere with intracranial self-stimulation, and there seems to be a general consensus that the interference is caused by a blockade of dopamine receptors in the brain [11,24]. This interpretation is supported by lesion studies in which destruction of dopaminergic neurons with 6-hydroxydopamine produces a long-term reduction in self-stimulation [2, 22, 23]. It is now recognized that several subtypes of dopamine receptors exist. D1 receptors are linked to adenylate cyclase activity, whereas D2 receptors are not [6,17]. Most of the neuroleptic drugs are antagonists at both receptor subtypes. Although other subtypes of dopamine receptors, i.e , D3 and D4, have been suggested, their pharmacological characteristics have not been determined.

Gallistel and Davis [13] conducted a comparative study of nine neuroleptic drugs and reported a high correlation between the relative affinity for the D2 receptor and drug-induced suppression of hypothalamic self-stimulation. Such results would suggest that brain-stimulation reward is mediated by neurons having D2 receptors There was, however, a notable exception. Haloperidol had a much lower affinity for the D2 receptor (K_i=1.5 nM) than did spiroperidol (K_i=0 25 nM), but these two drugs were effective in suppressing self-stimulation at similar dose ranges: haloperidol at 0 04–0.14 mg/kg and spiroperidol at 0 03–0 11 mg/kg.

There are several dopamine antagonists that show an extremely low affinity for D1 receptor For example, sulpiride is practically inactive at D1 receptor sites, having a K_i of 47,000 nM compared to a K_i of 190 nM for the D2 receptor [14]. Ferrer and coworkers [10] trained rats to make bar-pressing responses for stimulation of the medial prefrontal cortex and injected sulpiride at various dose levels. Sulpiride began to interfere with the animal's motility at 20 mg/kg IP, but it did not reduce the rate of responding until the dose was increased to 40 mg/kg IP Furthermore, intracranial injection of sulpiride into the vicinity of the electrode site in the prefrontal cortex did not interfere with self-stimulation. These findings are not in accord with the view that dopamine D2 receptor is critically involved in brain-stimulation reward.

SCH 23390, on the other hand, has a very high affinity for the dopamine D1 receptor (K_i=1.3–11 nM) compared to its affinity for D2 receptor (K_i=880 nM) [1, 4, 14] At doses between 0 11 and 2.1 mg/kg in the rat, SCH 23390 suppressed stereotypic behaviour produced by apomorphine [4,15], showing a dopamine antagonist action It had no effect on serum prolactin levels [15], which is considered to be under D2-receptor control [6]. Therefore, SCH 23390 is highly likely to have a competitive antagonist action at the D1 receptor sites but not at the D2 sites. In the present study, SCH 23390 and sulpiride were used to identify the receptor subtype of dopaminergic neurons involved in intracranial

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self-stimulation. Haloperidol, which blocks both D1 and D2 receptors, and metergoline, a serotonin receptor blocking agent, were tested for comparison

METHOD

Long Evans male rats (Charles River, Canada) were implanted, as previously reported [20], with stainless-steel bipolar electrodes into either the lateral hypothalamic area (LH), the ventral tegmental area (VT), or the dorsal raphe nucleus (DR). The stereotaxic coordinates [21] were as follows: A 5 4, L 1.7, V −2 3 for LH; A 2.6, L 1 2, V −3 4 for VT; and A 0 0, L 0 0 V −2.3 for DR

Spout Contact

The method of training and testing in a spout box has been reported previously [20]. After a 7-day recovery period, the rats were trained to receive brain stimulation by making body contact with a metal spout in a test box The animals generally used the snout or forepaw to make contact, but sometimes licked or bit the spout. Electrical stimulation was a train of square pulses having a pulse duration of 0.3 msec and a frequency of 100 pulses per sec. The train lasted as long as the contact was maintained up to a maximum of 0 5 sec The intensity of stimulation was adjusted for each animal, and a record of 30-min responding was examined periodically. If the response rates in three 10-min periods did not differ from their mean by 33%, the animal was trained to continue responding for an additional period of 60 min at the same stimulation intensity. A group of 10 rats were trained, 6 with LH electrodes, 2 with VT and 2 with DR electrodes

A stock solution of SCH 23390, 1 mg/ml free base, was prepared using 5% Tween-80 in physiological saline Haloperidol was prepared by diluting Haldol (McNeil) with distilled water Metergoline was dissolved in 2% ascorbic acid, and sulpiride was dissolved in 0 2 N acetic acid. After 30 min of baseline recording, animals were injected IP, and responding recorded for an additional 60 min.

The ED50 for SCH 23390 was determined in an additional group of 7 rats, all implanted with electrodes into the LH and trained in the spout box. The animals were injected with physiological saline and then progressively higher doses of SCH 23390: 0.01, 0.02, 0.04, and 0.08 mg/kg IP There was a minimum of 2 days between two consecutive injections. In each session, the total number of responses made in a 30-min period starting 10 min after injection was compared with the pre-injection rate and expressed in percentage for individual animals A dose response function was plotted for each animal, and a dosage just sufficient to suppress the response rate to 50% of the rate after saline injection (ED50) was calculated by interpolation.

Runway

Five of the rats used in the spout box, all with LH electrodes, were also tested in a runway 150 cm long and 10 cm wide, surrounded by 30 cm walls A guillotine door was placed 30 cm from one end, demarcating a starting box. The rat was placed in the starting box, and 15 sec later stimulated with a series of 10 priming trains. Each train consisted of 64 pulses at a frequency of 100 per sec, and each pulse was 0 3 msec in duration. The train was repeated every second for 10 times. The door opened 5 sec after the start of the last priming train, and the animal was allowed to run to make a contact with a spout on the wall at the other end of the runway.

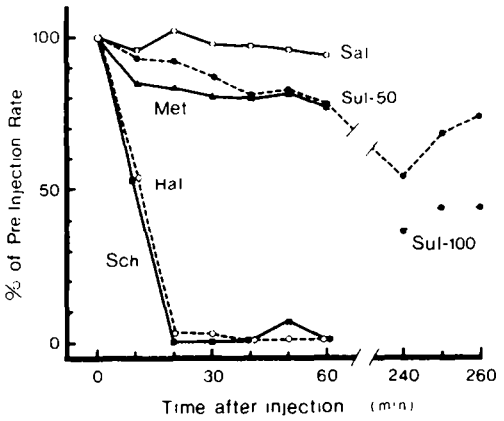

FIG 1 Changes in response rate after intraperitoneal injection of physiological saline (Sal), SCH 23390 0 05 mg/kg (SCH), haloperidol 0 25 mg/kg (Hal), and sulpiride 50 mg/kg (Sul-50) and 100 mg/kg (Sul-100), expressed as percent of pre-injection response rate The data are based on 10 rats with electrodes in the lateral hypothalamic area (n=6), ventral tegmental area (n=2), and dorsal raphe nucleus (n=2)

Regardless of the duration of contact, the first contact initiated a train of goal stimulation, which consisted of 0.3 msec pulses at 100 per sec. The number of pulses in the goal stimulation was either 4, 8, 16, 32, 64, or 128. The intensity of the goal stimulation was the same as the intensity of the priming stimulation, and adjusted for each animal in such a way that the animal's running speed attained a maximal asymptotic level with 64 and 128 pulses but not with 16 pulses The running speed was derived from the time interval between the door opening and spout contact and expressed in cm/sec Each rat was tested with 10 trials at each pulse number, first in an ascending order and then in a descending order. If an animal did not reach the spout within 20 sec, it was gently pushed to the end of the runway and given a train of goal stimulation. After a repetition of 4 no-run trials, the remaining trials were cancelled, and the minimal speed of 6.0 cm/sec was assigned to these trials. Mean speed of the last 5 trials was plotted to form a "reward summation function" [8]

On test days either physiological saline or SCH 23390 was injected 20 min prior to a test session Starting with a low pulse number an ascending and a descending series were completed in about 60 min. The locus of rise, defined as the number of pulses required to produce a speed which is 50% of the maximal speed [8], was calculated by interpolation. For each rat, the mean of two loci, one in the ascending and the other in the descending series, was determined in each test session.

RESULTS

In the spout box, the mean response rates per min (± standard error) in the 30-min period just prior to saline injection were: 77±3 6 for LH, 45±5.3 for VT, and 69±21 9 for DR. Following saline injection all animals returned immediately to the spout and continued responding for 60 min at rates which did not differ from the pre-injection rate. Drug-induced changes in response rate are depicted in Fig. 1 Haloperidol, 0.25 mg/kg IP suppressed self-stimulation completely 10 min after injection. The response rate in a

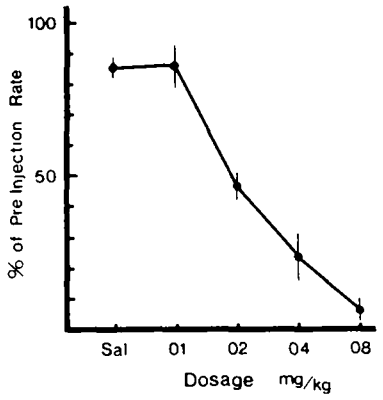

FIG 2 Dose-response function of SCH 23390 IP Vertical bars represent the standard error of the mean The data are based on 7 rats with electrodes in the lateral hypothalamic area

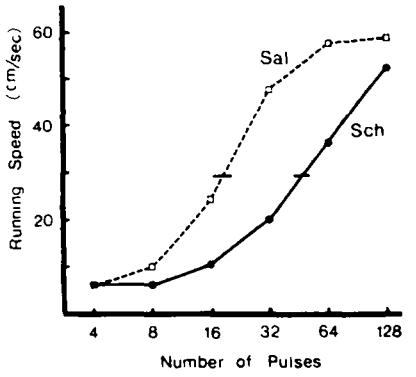

FIG 3. Reward summation functions after injection of physiological saline and SCH 23390 0.01 mg/kg IP Horizontal bars indicate the standard error of the mean for the locus of rise The data are based on 5 rats with electrodes in the lateral hypothalamus.

30-min period starting 10 min after injection was significantly less than the pre-injection rate ($t=6.607$, $p<0.01$).

SCH 23390, 0.05 mg/kg IP, produced a similar suppression ($t=8.616$, $p<0.01$). The activity of SCH 23390 was dose-related, and ED50 was 0.022 mg/kg IP (Fig. 2). When not responding to the spout, some animals stayed quietly in front of the spout, others turned away from the spout and some groomed. At no time did the animals sleep or freeze at the spout.

Metergoline, 5 mg/kg IP, and sulpiride, 50 mg/kg IP, were totally ineffective (Fig. 1). Since sulpiride is known to have a slow onset of action [27], 6 of the animals (2 each with electrodes in LH, VT, and DR) were returned to the spout box again 4 hours after injection. They were somewhat slower in responding but did not show any significant reduction in response rate ($t=2.264$) compared with the saline control. At 100 mg/kg IP, sulpiride produced dyscoordination, and the animals frequently slipped off the grid floor. However, they still managed to lie in front of the spout and to continue responding, though at significantly reduced rates ($t=4.472$, $p<0.01$). The results were similar with all electrode locations.

The results of the runway test are shown in Fig. 3 Following saline, the number of pulses required to produce a half maximal speed (locus of rise) was 20.1, whereas it was 52.0 after injection of SCH 23390 0.01 mg/kg IP This shift in the locus of rise was statistically significant $t=4.799$, $p<0.01$). The maximal speed was reduced from 58.5 cm/sec following saline to 52.5 cm/sec following the drug, but this decrease was not statistically significant ($t=1$ 972). After injection of SCH 23990 0.02 mg/kg, one of the rats completely stopped running, and one other rat stopped at 0.04 mg/kg. Those animals which ran, however, showed mean maximal speed of 57.2 cm/sec at 0.02 mg/kg and 49.5 cm/sec at 0.04 mg/kg, neither of which differed significantly from the speed after saline injection.

DISCUSSION

SCH 23390 and haloperidol suppressed self-stimulation of the LH, VT, and DR, whereas metergoline had no effect Sulpiride reduced responding only at a dose level that interfered with motor coordination. The suppression of bar-pressing by haloperidol is well known [29], and the present results assures that the spout contact method is sensitive enough to show the suppressive effect of the neuroleptic substance. Though SCH 23390 has some affinity for serotonin receptor (Ki=30 nM) [14], the suppression of self-stimulation by SCH 23390 cannot be attributed to serotonergic blockade because metergoline, at a dose which blocks tryptophan-induced hyperactivity [7] and suppresses self-stimulation of the habenula and median raphe [20] had no effect in the present experiment.

The dose of SCH 23390 required to suppress self-stimulation in the present study was similar to the dose required to suppress conditioned avoidance responses [15] and to antagonize the behavioural effects of amphetamine and apomorphine [4]. These finding suggest that the suppression of self-stimulation by SCH 23390 was through dopamine-receptor blockade Since SCH 23390 does not give rise to an increase in serum prolactin level [15], a D2 receptor response, it can be concluded that suppression of self-stimulation was mediated by blockade of D1 receptors.

The results of the runway experiment clearly indicate that the locus of rise had shifted after injection of SCH 23390 0.01 mg/kg IP. At this dose, the rewarding effect of stimulation was reduced without causing any other performance interference This method is one of the best in distinguishing the effect on reward from other interference on performance [18], but it does have a limitation At higher dose levels, where spout contact was completely suppressed, some animals did not run at all in the runway. Whether the failure to run was due to a complete loss of rewarding effect or to some other interference could not be determined Possibly an ultimate answer to this question will come from the use of a response requiring minimal physical activity. A preliminary report by Fantie [9] who used hippocampal theta waves as an operant response seems to suggest that SCH 23390 abolishes the rewarding effect of brain stimulation.

The failure of sulpiride to suppress self-stimulation cannot be attributed to poor penetration of the drug into the brain. Intraperitoneal injection of 100 mg/kg yields a concentration of approximately 2 μg/g in the hypothalamus [19], 50 mg/kg IP is sufficient to antagonize turning responses in-

duced by apomorphine in rats unilaterally lesioned in the striatum [12], and 20 mg/kg IP is enough to antagonize hyperactivity produced by amphetamine injected into the nucleus accumbens [5] Furthermore, none of these listed effects can be attributed to D1 receptor blocking, since sulpiride 50 mg/kg IP does not interfere at all with the increase in cerebral cyclic AMP produced by apomorphine [28] Therefore, it can be concluded from these data that the doses of sulpiride used in the present experiment were sufficient to block D2 receptors appreciably without affecting D1 receptors. Considering the effects of sulpiride together with the effects of SCH 23390, the present results suggest that D1 receptors and not D2 receptors are critical in producing the rewarding effect of brain stimulation in the LH, VT, and DR

The present findings are consistent with the report that self-stimulation of the prefrontal cortex, though involving dopamine transmission, does not involve D2 receptors [10] The present results, however, appear to be discrepant with the findings that the effective dose of neuroleptic drugs for the suppression of self-stimulation is correlated with the drug's affinity for D2 receptors and not D1 receptors. This discrepancy may have come from the fact that the *in vitro* affinity is not a sole determinant of a drug's effectiveness *in vivo*. A high-affinity drug may have little behavioural effect unless it is carried to the receptor sites across the blood-brain barrier On the other hand, a low-affinity drug may show a strong effect if it is accumulated in the effective region in the brain For example, haloperidol is known to reach brain concentrations 20-folds higher than blood levels [3] It is important to emphasize that the conclusion in the present study in favour of D1 receptors over D2 receptors has been drawn entirely from *in vivo* studies

There is growing evidence that D2 receptors are related to a certain type of schizophrenia, but the function of D1 receptors has remained unknown [25,26]. The present findings suggest that D1 receptors may be related to intracranial reinforcement of operant responses Various reinforcers, such as food, water, stimulant drugs and narcotics, have been reported to lose their reinforcing effect under the influence of neuroleptic drugs [30] Whether or not all of these effects can be attributed to the blockade of D1 receptor remains to be clarified by further experiments

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