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Apomorphine-Induced Facilitation of Intracranial Self-stimulation Following Dopamine Denervation of the Nucleus Accumbens

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STRECKER, R. E., D. C. S. ROBERTS AND G. F. KOOB. *Apomorphine-induced facilitation of intracranial self-stimulation following dopamine denervation of the nucleus accumbens.* PHARMAC. BIOCHEM. BEHAV. **17**(5) 1015–1018, 1982.—Two groups of rats were trained to lever press for intracranial self-stimulation (ICSS) from electrodes aimed at the posterior lateral hypothalamus or at the region of the locus coeruleus. Following stabilization of baseline responding using descending rate/intensity functions, bilateral 6-hydroxydopamine (6-OHDA) lesions to the nucleus accumbens (N.Acc.) were performed. Subsequent injections of apomorphine (SC) resulted in significant increases in self-stimulation in both lesion groups and significant decreases in self-stimulation in both groups of sham operated animals. These results indicate that the destruction of the dopaminergic terminals in the nucleus accumbens results in a "supersensitive" enhancement of the ICSS stimulating properties of apomorphine regardless of the electrode placement. Both lesion groups also showed a pronounced increase in locomotor activity in photocell cages following treatment with the same dose of apomorphine. These results complement previous work showing that dopamine destruction affects ICSS regardless of electrode placement and support the hypothesis that the midbrain dopamine systems have a general response enabling role in reinforced behavior.

Intracranial self-stimulation Apomorphine 6-Hydroxydopamine Nucleus accumbens Dopamine

SEVERAL studies have demonstrated that chronic neuroleptic treatment can result in increases in spontaneous and drug induced motor stimulation from dopamine agonists [7, 15, 17]. Similar but more pronounced results can be obtained from denervation of the terminals of the mesolimbic dopamine (DA) system using intracerebral injections of 6-hydroxydopamine into the region of the nucleus accumbens (N.Acc.) [9,12]. Termed "supersensitivity" this increased responsiveness to dopamine agonists is thought to reflect an increase in the number of DA receptors in the terminal regions of the DA systems [1,2].

Recent evidence has shown that the "supersensitive" response to DA agonists following chronic neuroleptic treatment could be extended to other behavioral responses such as a facilitation of ICSS [3]. In that study, a 3 day twice daily injection of pimozide resulted in an increase in ICSS following the termination of the pimozide treatment. Although these authors also showed a significant increase in stereotyped behavior following the pimozide treatment, suggesting the involvement of the nigrostriatal DA system, other studies have clearly shown increased apomorphine-induced locomotor activity following chronic neuroleptic treatment [15]. Unclear at this time is what role the mesolimbic DA system could have in the expresssion of the "supersensitive" ICSS response observed by Ettenberg and Milner [3].

The purpose of the present study was to determine whether N.Acc./6-OHDA treated animals, which show enhanced locomotor activity in response to apomorphine would also show an increase in their rate of responding for ICSS following apomorphine treatment. Additionally, considerable debate has centered on the question of the relative importance of dopamine and noradrenaline in mediating ICSS behavior at any particular brain site [8]. It was therefore of interest to determine whether any supersensitivity to the DA receptor agonist apomorphine might have differential effects depending on the site of the electrode. Two electrode sites were chosen: the posterior lateral hypothalamus (PLH) which contains DA and NE pathways and the region of the locus coeruleus (LC) which is thought to contain no major dopamine projections.

METHOD

Animals

The subjects were 32 male Wistar rats (Charles River) weighing 280–350 g at the time of electrode implantation.

Surgery

All rats had a single bipolar stainless steel electrode (0.2 mm diameter, Plastic Products Co., Roanoke, VA) implanted while under Chloropent anaesthesia (3.75 ml/kg) aimed either at the posterior lateral hypothalamus (PLH) with head flat, −4.0 mm posterior to bregma, 1.5 mm lateral to midline and 8.5 mm below skull at point of entry, or the locus coeruleus (LC): with head flat, −0.8 mm from ear bar zero, 1.1 mm lateral to midline and 7.4 mm below skull surface at lambda.

Apparatus

Five days following surgery the animals were allowed access to ICSS in 8 identical Plexiglas cages (30.5×18.0×30.5 cm) each containing one large metal lever (10×5 cm). A 300 msec train of 60 Hz constant current AC pulses was delivered to the brain with each lever press.

Procedure

Rats were normally allowed to learn the operant without shaping, but were shaped if necessary. During these daily 30 min preliminary training sessions, current levels were set at 30 μA for the LC rats and 25 μA for the PLH rats. These current levels were chosen to facilitate acquisition at each ICSS site with a minimum amount of shaping [11]. Two out of 15 LC rats required shaping, only one out of 16 PLH rats required shaping. Following 3 days of stable responding at a high rate (over 500 responses per session for the LC placement and 1,000 responses for the PLH placement) all rats were exposed to a decreasing series of 8 different current intensities. Currents ranged from 50 μA to 15 μA rms for the LC rats and from 40 μA to 5 μA rms for the PLH rats. Each current intensity was presented for 5 minutes and was signaled by white noise on, followed by a one minute time out period which was signaled by white noise off. The current level was decreased in 5 μA steps in each 5 successive minute period. Once stable levels of responding had been achieved (usually 5–10 days) the rats with each electrode placement were divided into sham and lesion groups (n=8 for each group, except for the sham LC group in which n=7). The groups were matched and balanced for baseline rates of responding.

Lesion Procedure

For surgery, the rats were anesthetized with Chloropent and pretreated with pargyline (50 mg/kg). The rats in the lesion group were bilaterally injected with 6-hydroxydopamine (6-OHDA) 8 μg/2 μl, (dosage expressed as the free base) dissolved in saline containing ascorbic acid (0.2 mg/ml). Injections were made through a 30 ga needle at a rate of 1 μl/3 min. The needle was left in place for 1 min following the infusion. The injection site as located histologically corresponded to the following anterior-posterior coordinates of König and Klippel stereotaxic atlas [10]: A +8920. For the PLH rats the actual coordinates for the lesion with the tooth bar +5 mm zero above interaural were +3.4 mm from bregma, ±1.7 mm lateral and −7.2 below dura. For the LC rats the actual coordinates were, with the tooth bar at −4.2 mm, +9.8 anterior from ear bar zero, lateral ±1.3 mm, +2.5 mm above ear bar zero. Sham lesioned rats were treated identically except that they were injected with 2 μl of vehicle not containing 6-OHDA. The two different coordinate parameters resulted from the experiments being conducted at different times. However, our results show an identical supersensitive locomotor response in the two groups and unpublished results in our laboratory indicate that both coordinate systems effectively block cocaine self-administration.

Beginning three days following the lesion all rats were again tested on the descending current series until responding stabilized. Two to three weeks post lesion all rats were treated with saline and then with apomorphine (0.1 mg/kg apomorphine HCl dissolved in saline, SC) 5 min before the ICSS session. Additionally, the rats were tested for supersensitivity to the locomotor activating effects of apomorphine (0.1 mg/kg) in photocell cages. Locomotor activity was measured in a bank of 16 Wahmann animal cages (40×25×20 cm) modified with 2 equally spaced horizontal infra-red photocell beams traversing the short axis 2 cm above the floor. Each interruption of either beam resulted in one count.

Statistics

ICSS results were subjected to a three factor analysis of variance (ANOVA) with repeated measures. Sham versus lesion groups formed the independent factor and drug and current intensity the repeated measures. Subsequent simple main effects tests were performed for individual comparisons of total session responding collapsed over current intensity, and drug effects for individual means comparisons between drug treatments at a given current intensity were analyzed using the paired t-test [20]. Locomotor activity scores were similarly analyzed using a two factor ANOVA with repeated measures. Sham versus lesion groups formed the independent factor and time the repeated measure.

At the conclusion of the experiment the rats were sacrificed by decapitation, and the brains were quickly removed and dissected on ice. The frontal cortex, nucleus accumbens, anterior corpus striatum and posterior corpus striatum were dissected from 2 mm slices uniformly cut with a Research Instruments Co. Cortical Slicer. Tissue samples were stored at −40°C until DA levels were determined using high pressure liquid chromatography with electrochemical detection [5].

RESULTS

As previously reported [7,9] 6-OHDA lesion of the N.Acc. was found to produce a supersensitive locomotor response to a low dose of apomorphine (0.1 mg/kg). The cumulative total beam interruptions for the 90 min post-injection revealed a highly significant increase in activity in the lesion groups (Sham PLH group and sham LC group: the mean totals ±S.E.M., 112±17 and 455±89, respectively; lesion PLH group and lesion LC group: the mean totals ±S.E.M., 1659±468 and 1746±311, respectively; PLH group: $F(1,14)=10.900$, $p<0.05$; LC group, $F(1,13)=14.307$, $p<0.05$).

In Figs. 1 and 2, ICSS responses are plotted as the number of responses per min in each 5 min period corresponding to each current intensity level. Overall analysis of variance revealed a significant Group × Drug interaction in both Groups, PLH: $F(1,14)=8.069$, $p<0.05$; LC: $F(1,13)=14.433$, $p<0.05$. Sham animals in both groups showed similar decreases in responding for ICSS following treatment with apomorphine, and this decrease was statistically significant in the PLH group over all current intensities, $F(1,14)=5.960$, $p<0.05$, simple main effects. ICSS rates in the sham LC animals also decreased following apomorphine injection

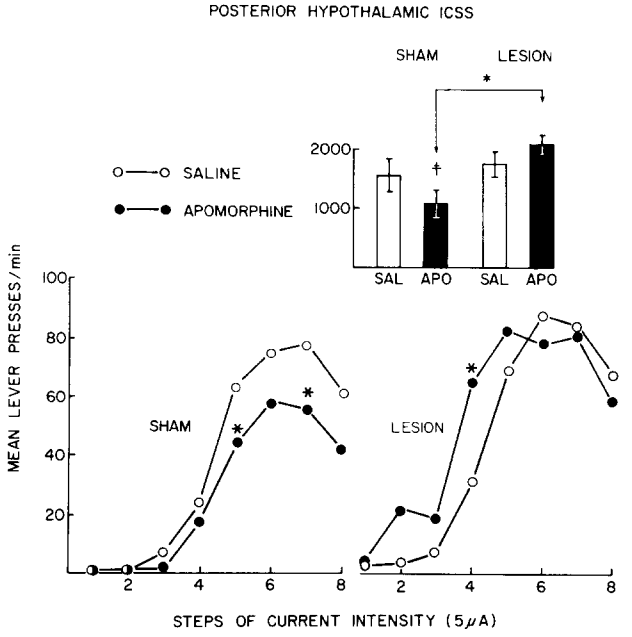

FIG. 1. Effects of apomorphine (0.1 mg/kg SC) on intracranial self-stimulation in rats with electrodes aimed at the posterior hypothalamus. Values in the large bottom panel are the means for each group for 5 min periods at each step of current intensity in a descending series of 8 steps. Values in the insert represent the mean±S.E.M. of the total number of the lever presses for the duration of the session. Lesion refers to the 8 rats receiving a 6-OHDA lesion to the region of the nucleus accumbens. *Significantly different from the sham group, $p<0.05$, ANOVA, simple main effects. ‡Significantly different from the corresponding saline injection, $p<0.05$ ANOVA, simple main effects. *In the lower figure refers to a significant difference between drug treatment at any given current intensity, $p<0.05$, paired t-test following ANOVA, see text. Sham animals=8.

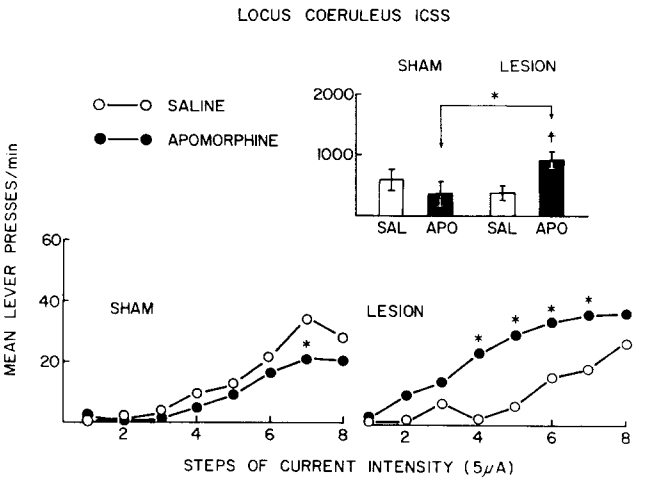

FIG. 2. Effects of apomorphine (0.1 mg/kg, SC) on intracranial self-stimulation in rats with electrodes aimed at the region of the locus coeruleus. Legend as in Fig. 2. Values in the large bottom panel are the means for each group for 5 min periods at each step of current intensity in a descending series of 8 steps. Values in the insert represent the mean±S.E.M. of the total number of the lever presses for the duration of the session. Lesion refers to the 8 rats receiving a 6-OHDA lesion to the region of the nucleus accumbens. *Significantly different from the sham group, $p<0.05$, ANOVA, simple main effects. ‡Significantly different from the corresponding saline injection, $p<0.05$ ANOVA, simple main effects. *In the lower figure refers to a significant difference between drug treatment at any given current intensity, $p<0.05$, paired t-test following ANOVA, see text. Sham animals n=7.

when compared to saline injection, but only at the high current intensities (Group × Drug × Current interaction, $F(7,91)=5.847$, $p<0.05$, see asterisks in Fig. 2).

In contrast the animals with N.Acc. lesions showed an increase in responding for ICSS when treated with apomorphine, and this increase was statistically significant in the LC group over all current intensities, $F(1,13)=18{,}046$, $p<0.05$, simple main effects. ICSS rates in the PLH lesion rats also increased following apomorphine injection when compared to saline injection, but only at the middle current intensities (Group × Drug × Current interaction, $F(7,98)=2.955$, $p<0.01$, following log transformation), see asterisks in Fig. 1. The log transformation revealed a significant three way interaction here whereas the untransformed data did not. This was attributed to a large difference in variance within each cell of current intensity in the raw data. Because of this and the repeated measure, i.e., the same animals received saline and then apomorphine; a paired t-test was used for post-hoc individual mean comparisons.

Further, direct comparisons of the sham versus lesion animals under the influence of apomorphine as in the analysis of the locomotor activity showed that for both the PLH and LC groups the total number of responses for the animals with lesions is significantly larger than that for the animals with sham lesions, PLH: $F(1,20)=10.63$, $p<0.05$; LC: $F(1,13)=18.046$, $p<0.05$.

Examination of the DA content of the N.Acc. for the PLH group using high performance liquid chromatography revealed the lesioned group to have an average of 64% less DA in the N.Acc. than the sham operated control rats. The mean levels for sham animals for N.Acc. were 33.12±2.74 ng/mg protein and for the lesion rats 11.16±2.76 ng/mg protein; this difference was statistically significant, $t(11)=6.636$, $p<0.05$.

DISCUSSION

The results reported in the present study support the hypothesis that the supersensitivity to apomorphine following destruction of dopaminergic terminals is a generalized phenomenon, here found to enhance responding for ICSS in addition to the previous reports of increased locomotor and DA receptor binding [2,9]. In this study, a low dose of apomorphine significantly increased responding for ICSS at both posterior hypothalamic and locus coeruleus sites in the rat with lesions to the nucleus accumbens. At the same doses responding for ICSS in the rats with sham lesions was actually significantly decreased. These results are consistent with previous reports showing that apomorphine in general has a depressing effect on ICSS in normal rats [13,19], but can reinstate responding after catecholamine depletion with α-methyl-p-tyrosine treatment [16].

Although the supersensitive ICSS response to apomorphine may reflect an alteration in reward mechanisms, a hypothesis involving nonspecific activation as reflected in the supersensitive locomotor stimulation effects of apomorphine cannot be ruled out. In fact, Roberts *et al.* [14] found no change in self administration of apomorphine following similar lesions of the N.Acc. Nevertheless, it could be that ICSS has a less restrictive response profile than does self-administration. For example, the increase in the rewarding value of apomorphine in rats self-administering apomorphine should be reflected in a decrease in responding, not in an increase. Whether or not the facilitation of ICSS seen with apomorphine in N.Acc. dopamine denervated rats is a facilitation of reward is an interesting question and more work characterizing other situations where apomorphine can impart reinforcing properties may be useful in resolving this issue. For example, in a recent study using place preference instead of operant self-administration to reflect the rewarding value of apomorphine, rats showed a significant shift to the left of the dose-response relationship following similar N.Acc. lesions [18].

However, an issue that does appear to be resolved by the present paper is the notion that the action of a receptor agonist in ICSS must necessarily be interpreted as an action on the neurons directly stimulated by the electrical current. For example, ICSS at both PLH and LC sites was facilitated by apomorphine following N.Acc. denervation clearly demonstrating that the DA receptor activation here is a more general facilitation of responding for ICSS, and is not specific for "dopaminergic" sites. Similar non selective enhancement of ICSS has been observed by others following termination of chronic dopaminergic receptor blockade [4]. The present study provides further evidence against the concept of "local" catecholamine reward circuits [6] and suggests a much more general role for DA in reinforced behavior.

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