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Effect of Chronic Apomorphine on the Development of Denervation Supersensitivity

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ROBIN, M., C. FORLER AND M. G. PALFREYMAN. *Effect of chronic apomorphine on the development of denervation supersensitivity*. PHARMACOL BIOCHEM BEHAV 22(4) 547–551, 1985.—We hypothesised that it would be possible to prevent the development of post-synaptic dopamine receptor supersensitivity to 6-hydroxydopamine lesions of the nigro-striatal tract in rats if they were constantly infused with the dopamine agonist apomorphine. Using osmotic minipumps to infuse apomorphine for 15 days in unilaterally lesioned rats, it was possible to delay the development of supersensitivity of the lesioned side for 9 days but not to prevent its eventual appearance. At the same time, evidence for the development of subsensitivity of presynaptic dopamine receptors of the intact side following chronic infusion of apomorphine was inferred from the production of rotations directed towards the lesioned side.

Dopamine receptor supersensitivity Apomorphine induced rotational behaviour 6-Hydroxydopamine lesions Nigro-striatal dopamine tract

CHANGES in receptor sensitivity may be observed in response to removal of the presynaptic input [19] and in response to chronic agonist [1, 9, 11, 13, 15, 16, 24] or antagonist exposure [4, 10, 18]. This adaptive phenomenon is now recognised as being of considerable importance in the long term effects of drugs as well as contributing to our understanding of the pathophysiology of several disease processes.

In the CNS, the rotational model originally described by Ungerstedt [20] has been extensively used to investigate dopaminergic supersensitivity after unilateral denervation of the neostriatum. We hypothesised that if the adaptive response of the denervated post-synaptic receptors was solely due to loss of the transmitter, then "replacement" of the transmitter by chronic infusion of a dopamine agonist would prevent the supersensitivity from developing. This report describes our attempts to verify this by a chronic infusion of apomorphine in unilaterally 6-hydroxydopamine-lesioned rats.

METHOD

Animals

Male Sprague-Dawley rats (Charles River, France), weighing 280–320 g on the day of lesion, were used. Rats were housed individually in a humidity- and temperature-controlled room with a 12 hr light-dark cycle (6 a.m.–6 p.m.).

Lesions

Rats were lesioned unilaterally by injecting 6-hydroxydopamine (6-OHDA, HBr; Sigma) in the right median forebrain bundle using the following procedure: Rats were pretreated with 25 mg/kg IP of desmethylimipramine HCl (Ciba-Geigy) 30 min before injection of the neurotoxin in order to block the uptake of 6-OHDA into noradrenergic fibers and thus produce selective degeneration of dopaminergic neurones [3,8]. Surgery was then carried out under halothane/N_2O/O_2 anaesthesia, in a stereotaxic frame (Kopf Instruments, Tujunga, CA). 6-Hydroxydopamine was dissolved in saline containing 0.2% (w/v) l-ascorbic acid and 8 μg in 2 μl were injected slowly over 5 1/2 min at the coordinates A=+3.8, L=+1.5, V=−3.5 [12]. To facilitate recovery of the rats after the lesion, food was made available on the cage floor and a bottle of water was provided in addition to the normal food and water delivery systems.

Behavioural Studies

Chronic apomorphine infusion. Apomorphine HCl, dissolved in distilled water (10 mg/ml) containing 0.2% (w/v) l-ascorbic acid was used. Such a solution was found to remain fully pharmacologically active for more than 4 weeks when kept in the dark at 37°C. This solution was delivered at a rate of 10 μl/hr using Alzet® 2 ML 1 osmotic pumps (Alza, Palo Alto, CA) implanted subcutaneously immediately after brain lesioning. The pumps were removed on day 8 and immediately substituted by new pumps that had been previously incubated in saline at 37°C so that drug delivery was not discontinued. The second pumps were left in place for a further 7 days at which time they were removed. The normal

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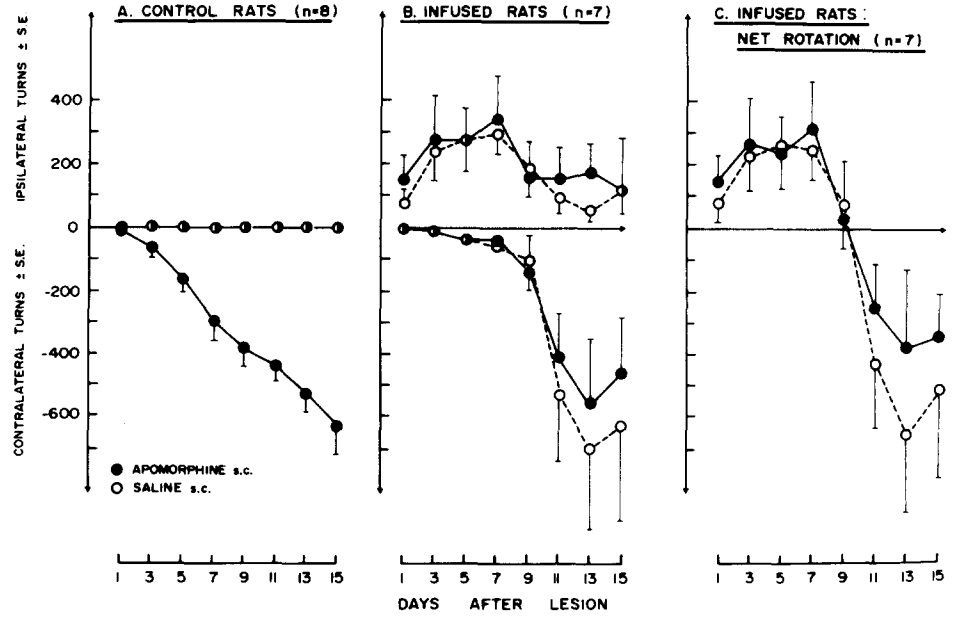

FIG. 1. Rotational behaviour to subcutaneous saline or apomorphine (0.2 mg/kg) challenge in A: 6-OHDA lesioned control rats and B: in 6-OHDA lesioned rats infused with apomorphine (100 μg/hr) for 15 days. Panel C shows the net rotations calculated from panel B by subtracting the minor directional component. Rotation is shown in the ipsilateral and contralateral direction for the 90 min observation period.

functioning of all pumps was *a posteriori* checked by measuring the remaining amount of solution after their removal. In some instances, the remaining content of the pumps were pooled and, after appropriate dilution, injected into naive 6-OHDA-lesioned rats to provide an additional control of drug stability. The concentration of apomorphine in the pumps was chosen to give a rotational response similar to the submaximal rotation produced by 0.2 mg/kg SC apomorphine (see Biochemical studies below). Control rats were lesioned with 6-OHDA at the same time but not implanted with pumps. Preliminary experiments had shown that rats implanted with pumps containing saline behaved in exactly the same way as rats bearing no pumps.

Recording of rotation. A set of four automated rotometers was used which allowed the recording of both clockwise and anticlockwise rotations greater than 180°. Rotations were recorded on alternate days until 15 days after lesioning commencing on day 1, 24 hr after operation. On each day of observation animals were injected with saline (1 ml/kg, SC) and rotations counted every 5 min over the following 90 min period. Apomorphine (0.2 mg/kg, SC) was then injected and a further 90 min recording made.

Biochemical Studies

Biochemical studies were conducted 5 days after 6-OHDA when maximal behavioural differences between control and apomorphine infused animals were evident.

Treatment of rats. A group of 15 rats was lesioned with 6-OHDA as described above then randomly divided into 3 equal groups. The control group was not otherwise treated; the second group was implanted SC with a pump delivering 100 μg/hr of apomorphine for the 4 hours preceding death and is referred to as the acute apomorphine group; the third group was implanted with pumps immediately after the lesioning procedure as described for behavioural studies and was thus infused with 100 μg/hr of apomorphine for 5 days before being decapitated. This group is referred to as the chronic apomorphine group.

Biochemical measurements. The three groups of rats were decapitated 5 days after the 6-OHDA lesion, brains were removed and the left and right striata rapidly dissected on an ice-cold plate then frozen at −80°C. Assays of dopamine (DA), homovanillic acid (HVA), dihydroxyphenylacetic acid (DOPAC) and norepinephrine (NE) were conducted using high performance liquid chromatography with electrochemical detection as previously described [22].

RESULTS

Behavioural Studies

Lesioned rats not implanted with pumps (control rats) displayed the expected behaviour [20] (Fig. 1A). Thus, when saline was injected, no consistent rotation was observed (less than 10 ipsilateral turns/90 min on any day of recording), while following apomorphine injection, rotation contralateral to the lesion was observed from day 3 and increased gradually in intensity up to the end of the experiment (day 15). No ipsilateral rotation was seen after the acute challenge with apomorphine in these animals.

Rats chronically infused with apomorphine behaved quite differently (Fig. 1B). When these animals were placed in the rotometer they rotated spontaneously. Subcutaneous injections of saline did not modify this rotational behaviour. More noteworthy, following SC injection of apomorphine rotation

TABLE 1

EFFECT OF 6-OHDA LESION IN THE CONTROL AND TREATED GROUPS

	Intact side (left)	Lesioned side (right)	% Change	Significance
Control Group				
DA	7582 ± 136	469 ± 119	−94%	<0.001
DOPAC	1723 ± 227	164 ± 36	−90%	<0.01
HVA	738 ± 41	35 ± 6	−95%	<0.001
NE	153 ± 73 (n=4)	94 ± 40 (n=4)	−39%	N.S.
Acute Apomorphine Group				
DA	6242 ± 636	218 ± 33	−97%	<0.001
DOPAC	1376 ± 121	174 ± 17	−87%	<0.001
HVA	484 ± 18	28 ± 2	−94%	<0.001
NE	191 ± 16	83 ± 14	−57%	<0.01
Chronic Apomorphine Group				
DA	4430 ± 347	166 ± 42	−96%	<0.001
DOPAC	1481 ± 154	130 ± 10	−91%	<0.005
HVA	482 ± 25	26 ± 3	−95%	<0.001
NE	169 ± 13	93 ± 13	−45%	<0.01

% Changes are calculated using the intact side as a reference and significance using the paired Student "*t*" test (two-tailed). n=5 except where shown. Values are expressed in ng/g wet tissue ± SEM.

was not different from the spontaneous or saline challenged rotation (Fig. 1B). The rotation in the rats chronically infused with apomorphine differed qualitatively from the rotation produced by injections of apomorphine in animals bearing a 6-OHDA lesion but not infused with apomorphine (compare Figs. 1A and 1B). Thus, in the apomorphine-infused animals ipsilateral rotation was seen from day 1 following saline or apomorphine challenge which increased in intensity to reach a maximum on day 7. The ipsilateral rotation then declined but was still present on day 15. Practically no contralateral rotation was observed up to day 7; thereafter, clear contralateral rotation developed over the following days. Thus, from day 9 the rotational behaviour following either saline or apomorphine injections consisted of bursts of alternating ipsi- and contralateral rotation. Figure 1C illustrates the predominant direction of rotation obtained by subtracting the minor component. In contrast to the situation seen in control lesioned rats (see Fig. 1A), contralateral rotation did not become dominant until day 9.

In complementary experiments pumps were removed surgically on days 8 and 15 and the recording resumed 1, 3 and 5 days later. These rats no longer rotated after saline. After 8 days infusion with apomorphine, an apomorphine challenge (0.2 mg/kg, SC) 1 day later produced only slight contralateral rotation (110±30 turns/hr) compared to the non-infused animals which rotated at 410±40 turns/hr following SC apomorphine. At 3 and 5 days after removal of the pumps, apomorphine challenge produced progressively greater contralateral rotations to attain, by day 13, a similar rate to that seen in non-infused animals. On the other hand, after 15 days of apomorphine infusion followed by 3 or 5 days without the pumps, apomorphine challenge produced a pure contralateral rotation that was 2–3 fold more intense than that seen on day 15. In contrast, sham-lesioned and non-lesioned rats infused with apomorphine (100 μg/hr) for 15 days did not show consistent rotation when challenged with either saline or apomorphine.

Biochemical Studies

Verification that the animals used in the biochemical studies were representative of those used for the behavioural studies was obtained in the following way: on day 4 after lesion the control lesioned group was placed in the rotometers and displayed no rotation. On day 5 after lesion, the acute apomorphine group was placed in the rotometers 15 minutes after implantation of the pumps. These animals displayed vigorous contralateral rotation (708±200 turns/90 min). On day 4 after lesion, rotation in the chronic apomorphine group was recorded and showed intense ipsilateral rotation (369±91 turns/90 min).

Comparison between the intact and lesioned striata of the three groups. In control lesioned rats, killed 5 days after lesioning, the three dopaminergic parameters were reduced by 90% or more on the lesioned side (Table 1, upper part). Norepinephrine levels in the lesioned striata of the control rats were not significantly altered.

In rats infused acutely (4 hr) or chronically (5 days) with apomorphine, the dopaminergic parameters were reduced on the intact side compared to controls and in these animals norepinephrine reduction on the lesioned side reached statistical significance (Table 1, middle and lower parts). Reference to Table 1 also shows that chronic infusion of apomorphine produced a more pronounced decrease of the dopamine concentrations on the intact side than acute apomorphine infusion (−29%, $p<0.05$).

DISCUSSION

Our experiments were designed to test the hypothesis that it would be possible to prevent the developement of dopaminergic supersensitivity in 6-OHDA-lesioned rats by constantly supplying a DA agonist. A similar approach, using the model of neuroleptic-induced supersensitivity, has been successfully tested in both behavioural and biochemical studies [2, 5, 6, 7, 14, 23]. To our knowledge, this has not been attempted in the case of supersensitivity induced by unilateral DA denervation, a model that allows the simultaneous observation of the events taking place in both the denervated and intact sides of the brain.

Our results show that denervation supersensitivity developed as expected [20] in the control rats not infused with apomorphine. These rats turned contralaterally to the lesion when challenged subcutaneously with apomorphine. This rotation increased gradually from day 3 to day 15, consistent with the progression of receptor supersensitivity as described by Ungerstedt [20]. In agreement with Ungerstedt [20] almost no ipsilateral rotation was seen in these rats and saline challenge was without effect.

In striking contrast, rats infused chronically with apomorphine from the day of lesioning behaved quite differently. First, these animals displayed rotational behaviour presumably reflecting the effects of the infused apomorphine. Second, rotation following subcutaneous challenge with apomorphine did *not* differ significantly in direction or intensity from that seen following subcutaneous saline injections. The rotational behaviour arising from the infused apomorphine pump is probably near to maximal since the injected apomorphine produced no additional effect.

These conclusions are supported by the observations that following surgical removal of the pumps (i.e., after 15 days of apomorphine infusion) rotation stops immediately. Furthermore if 3 days later these animals are challenged with saline they do not rotate spontaneously but they do display intense contralateral rotation to a subcutaneous challenge with 0.2 mg/kg of apomorphine indicating that supersensitivity is clearly established at this time. However, if pumps were removed after 8 days, apomorphine challenge 1 day later produced only slight contralateral rotation. This contrasts with the intense rotation seen in lesioned rats not infused with apomorphine. In addition, rats lesioned with 6-OHDA and then 5 days later infused with 100 μg/hr apomorphine turned vigorously (472±33 turns/hr) in the contralateral direction.

From the foregoing we must consider the turning behaviour in terms of the infused apomorphine producing the observed effects. Rats bearing a 6-OHDA lesion and infused with apomorphine from day one do not develop contralateral rotations until the 9th day after lesioning. This contrasts with the effects in rats not infused with apomorphine until day 5 after the lesion (see Biochemical studies, Results section) where intense contralateral rotation is observed to a subsequent infusion of apomorphine. Since contralateral rotation is a consequence of the development of post-synaptic supersensitivity we can conclude that continuous infusion of apomorphine prevents the development of this supersensitivity. Nevertheless supersensitivity does develop even in the face of continued apomorphine infusion after about 9 days although the development of supersensitivity appears to level off between the 13th and 15th day of infusion. This is true supersensitivity to apomorphine since removal of the pumps at day 15 followed 2 days later by an apomorphine challenge shows clear evidence of supersensitivity. This interpretation of the results needs a certain circumspection since during the first 9 days of apomorphine infusion there is a marked ipsilateral rotation which could conceivably "mask" an underlying supersensitivity. This is unlikely, however, because after the 9th day when clear cut contralateral rotation is developing there is still definite evidence of intermittent bursts of ipsilateral rotation. Clearly, ipsilateral *and* contralateral responses can occur in apomorphine infused animals. Furthermore, removal of the pumps after 8 days followed by apomorphine challenge 1 day later shows a considerably reduced sensitivity to apomorphine compared to non-infused animals.

The appearance of *ipsilateral* rotations during the first 7–9 days in the apomorphine infused animals is an interesting observation since it was never observed in the control lesioned animals. The ipsilateral rotation is robust, reaching 246±60 turns/hr and is still evident, although less marked, even after 15 days of infusion with apomorphine.

Ipsilateral rotations suggest that the intact side is more sensitive than the denervated side to the infused apomorphine. We doubt that this imbalance results from a decreased sensitivity of the *lesioned* side since ipsilateral rotations do not disappear when the lesioned side has apparently become supersensitive. Thus, the increased sensitivity of the intact side may be the underlying mechanism and could arise from effects on presynaptic receptors or from modifications of the sensitivity of the post-synaptic receptors on the intact side. This latter explanation is unlikely because on removal of the pumps for two days a subcutaneous injection of apomorphine produced intense *contralateral* rotation. Furthermore, changes in post-synaptic receptor sensitivity usually take time to develop and then persist for a long time [10]. Ipsilateral rotations are evident within 24 hours of starting the apomorphine infusion.

Although it is not at all clear why the rats rotate in an ipsilateral direction for the first 7 days it is conceivable that the infused apomorphine is acting presynaptically on the intact side to desensitize the presynaptic autoreceptors. This must occur rapidly. The reduced responsiveness of these receptors will mean that the depressant effect of the exogenous agonist, apomorphine, on dopamine release will be reduced together with a reduced feed-back control of dopamine release produced by the endogenous dopamine. The increased release of endogenous dopamine might then augment the effect of the exogenously supplied apomorphine on the post-synaptic receptors and drive the rotation in an ipsilateral direction. This presynaptic control cannot occur on the lesioned side. For this hypothesis to be tenable it is necessary to assume that the infused apomorphine is preventing the post-synaptic receptors on the lesioned side from developing their normal supersensitive response to denervation. This speculation gains further support from the studies of Müller and Seeman [11] in intact rats treated chronically with apomorphine and amphetamine where decreased [^{3}H] apomorphine binding (probably to presynaptic receptors) without changes in [^{3}H] haloperidol binding to the post-synaptic receptor was reported. Furthermore, numerous authors [1, 9, 11, 13, 15, 16, 24] have reported a paradoxical increase in sensitivity of animals to the behavioural effects of dopamine agonists in intact animals treated chronically with several different dopamine agonists, effects which are explained by a desensitization of presynaptic receptors.

Nevertheless, one would predict that a decrease in sensitivity of the presynaptic dopamine receptors would be re-

flected in an increase in the release of dopamine. However, this was not observed in determinations of dopamine metabolites on the intact side. In fact, there was a reduction in the concentration of dopamine, DOPAC and HVA on the intact side following either acute or chronic (5 days) infusion of apomorphine, an observation in agreement with Springer *et al.* [17]. This decrease in dopamine may be a consequence of inhibition of tyrosine hydroxylase [21] and is partially reinforced by the more marked decrease in norepinephrine seen following apomorphine infusion. It will await more detailed experiments before a clear explanation of this paradoxical ipsilateral rotation can be obtained. Our data are, however, consistent with the idea that it is possible to delay the development of post-synaptic denervation supersensitivity with a chronic infusion of the agonist, although clearly it has not been possible to prevent its eventual development.

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