Evidence for a receptor-mediated feedback control of striatal tyrosine hydroxylase activity

WE have previously reported that cutting the nigrostriatal dopamine-carrying axons unexpectedly results in a transient increase in the rate of tyrosine hydroxylation in the rat forebrain (Carlsson, Kehr & others, 1972). It was suggested that striatal tyrosine hydroxylase activity is controlled via dopamine receptors at the synaptic cleft: when the impulse flow is interrupted by axotomy, the concentration of dopamine in the synaptic cleft decreases, and the ensuing reduction of dopamine receptor activity gives rise to a feedback activation of tyrosine hydroxylase, located in the striatal dopamine-carrying nerve terminals.

The experiments now reported were made to test the above hypothesis. We argued that stimulation and blockade of dopamine receptors should result in inhibition and activation, respectively, of striatal tyrosine hydroxylase activity.

Male Sprague-Dawley rats, 210–340 g, were used. Axotomy of the nigrostriatal dopamine fibres was performed under ether anaesthesia on the left side by means of a transverse cerebral hemisection, as previously described (Bédard, Carlsson & Lindqvist, 1972). At the same time (or in some experiments after 1 h) the aromatic amino-acid decarboxylase was inhibited by an intraperitoneal injection of NSD 1015 (3-hydroxybenzylhydrazine HCl, 100 mg/kg). The animals were decapitated 30 min after the injection. The forebrains were analysed for dopa (Kehr, Carlsson & Lindqvist, 1972) and dopamine (Atack, to be published).

For a direct activation of dopamine receptors, apomorphine HCl, 15 mg/kg, was injected intraperitoneally 7 min before the transection, and for blockade of these receptors haloperidol was given 1 h before the transection (5 mg/kg intraperitoneally, for references see Andén, Carlsson & Häggendal, 1969). In some experiments both agents were given to the same animals.

The levels of dopa in the forebrains are given in Fig. 1. As previously reported, inhibition of the aromatic amino acid decarboxylase causes the accumulation of dopa, which cannot be detected in the normal brain. This accumulation appears to be a useful indicator of the rate of tyrosine hydroxylation (Carlsson, Davis & others, to be published). In animals transected and treated simultaneously with NSD 1015, the

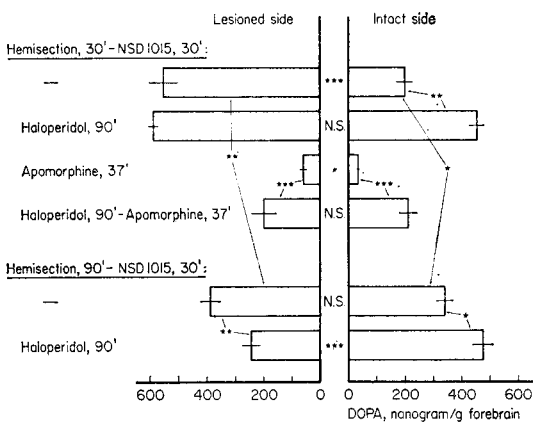

FIG. 1. Receptor-mediated feedback control of dopa synthesis in rat forebrain. The time interval (in min) between treatments and death are indicated in the Figure. The means of forebrain dopa levels ± s.e. on the lesioned (left) and intact side are shown. The number of experiments, each comprising 3 pooled forebrains, is 4, except for the group treated with haloperidol plus hemisection, 30 min, where n = 2. *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$, N.S. = not significant.

accumulation of dopa, induced by the decarboxylase inhibitor, was much more pronounced on the lesioned side than on the control side (the same data as reported by Carlsson & others, 1972). This accumulation was markedly inhibited on both sides by apomorphine. Haloperidol had the opposite effect on the intact side but had no significant effect on the lesioned side. However, if both drugs were given, the inhibitory effect of apomorphine on dopa formation was markedly antagonized by haloperidol, this effect being evident on both sides.

These observations support the hypothesis that the striatal tyrosine hydroxylase activity is controlled via the activity of dopamine receptors in the synaptic cleft. The transection by itself should reduce the dopamine concentration at receptor sites to a very low level by depriving the striatal dopamine system of its impulse flow. Blockade of the dopamine receptors by haloperidol cannot be expected to cause any effect on the lesioned side, since there is hardly any agonist present in the synaptic cleft. On the intact side, however, the expected increase in dopa formation was observed. Activation of dopamine receptors by apomorphine caused the expected inhibition of dopa formation on both sides, and this inhibition could be antagonized by receptor blockade: now the receptor-blocking agent could act on both sides, since a receptor-activating agent was present also on the lesioned side.

Fig. 2 shows the corresponding dopamine values. After hemisection and simultaneous NSD 1015 treatment the level was slightly lower on the intact than on the lesioned side, probably due to impulse-induced release and metabolism. The difference between the two sides was more pronounced after haloperidol but was reduced to an insignificant value after apomorphine; these data are in accordance with earlier observations showing that haloperidol and apomorphine stimulate and retard dopamine turnover, respectively, probably owing to a receptor-mediated feedback mechanism influencing the impulse-induced release and metabolism (see Andén & others, 1969). In agreement with this interpretation, a difference between the two sides again became apparent when the receptor stimulating action of apomorphine was partially blocked by pretreatment with haloperidol.

We had insufficient material to allow any definite conclusions about the differences in dopamine levels between different treatment groups 30 min after hemisection.

In some experiments the decarboxylase inhibitor was not given until 1 h after the transection. Then, in contrast to the results reported above, the accumulation of dopa was not significantly higher on the lesioned side in animals treated with NSD

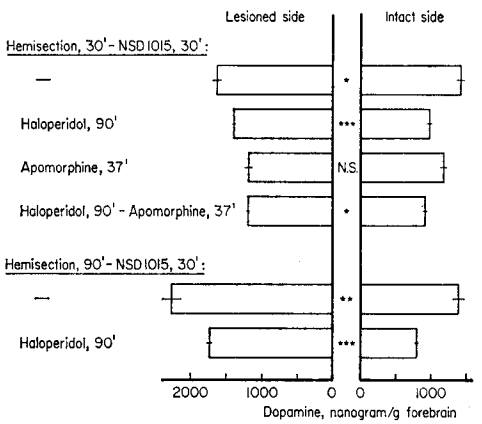

FIG. 2. Receptor-mediated feedback control of dopamine turnover in rat forebrain. The same animals as in Fig. 1. The difference between hemisection, 30′-NSD 1015, 30′, and hemisection, 90′-NSD 1015, 30′, lesioned side, is statistically highly significant ($P < 0.001$). For further explanations, see Fig. 1.

1015 alone. This may be due to feedback inhibition of tyrosine hydroxylase induced by the dopamine rapidly accumulating on the lesioned side (cf., Andén, Bédard, & others, 1972). Haloperidol, given at the time of transection, accelerated dopa formation on the intact side but, surprisingly, had the opposite action on the lesioned side. No explanation can as yet be offered for this apparent inhibitory action of haloperidol on the tyrosine hydroxylase activity on the lesioned side. There might be an interaction between haloperidol and the feedback inhibition induced by the accumulating dopamine.

After hemisection and NSD 1015 treatment the animals showed no consistent asymmetries. However, additional treatment with haloperidol induced turning towards the intact side, and after apomorphine to the lesioned side. The combined treatment with haloperidol and apomorphine caused turning towards the intact side. These observations, which are in agreement with those reported by Andén, Dahlström & others (1966) and Andén, Rubenson, & others (1967) indicate that the striatal dopamine receptors were indeed efficiently blocked and stimulated by haloperidol and apomorphine, respectively.

In conclusion, the present data indicate that receptor-mediated feedback mechanisms exist which control the rate of tyrosine hydroxylation as well as the turnover of dopamine in the rat forebrain. The synthesis rate can apparently be influenced by changes in dopamine receptor activity even in the absence of an impulse flow, as indicated by the observations made on the lesioned side after haloperidol and apomorphine treatment. On the other hand, the feedback control of dopamine turnover seems to depend to a considerable extent on the impulse flow, as earlier emphasized by Andén, Corrodi & others (1971). The mechanism by which dopamine synthesis and turnover can be controlled by dopamine receptor activity, is not clarified by the present observations. If the receptors involved are located postsynaptically, a transsynaptic messenger may be postulated. If presynaptic dopamine receptors exist at the synaptic clefts, the mediator involved might operate entirely intraneuronally.

Nybäck & Sedvall (1971) and Nybäck (1972) observed that the accumulation of [^{14}C]dopamine formed from [^{14}C]tyrosine in the rat striatum was accelerated by chlorpromazine. However, this effect was abolished by cutting the nigrostriatal dopamine axons. The authors concluded that the synthesis-accelerating action of chlorpromazine was mediated via an increased nerve impulse flow. Our data show, however, that the stimulating action on dopamine synthesis induced by another neuroleptic agent, i.e. haloperidol, can be demonstrated also after cutting the nigrostriatal dopamine axons, provided that an agonist is present at the dopamine receptor sites. Whether this is true also of chlorpromazine, remains to be elucidated.

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Kinin-like substances in saliva of larvae of wasps and hornets

A wasp or hornet larva touched on the head emits a drop of saliva which is a gland secretion and is known to contain organic nutrients, particularly carbohydrates (Maschwitz, 1965; Ikan & Ishay, 1966; Ishay & Ikan, 1968). A drop ranged in volume from 0·001–0·010 ml.

Saliva from 2500 larvae of *Vespa orientalis* was collected in a cold atmosphere and stored at −20° until added (0·05 ml) to guinea-pig isolated ileum or rat uterus (0·5 ml) preparations maintained in a 10 ml organ bath filled with either Tyrode solution at 35° or de Jalon solution at 28° respectively. Contractions were recorded with a transducer connected to a polygraph. Saliva was allowed in contact for 1 min and then the preparation was washed twice.

The saliva evoked contraction of both preparations (Fig. 1). Activity was not depressed after pretreatment with lysergic acid, mepyramine maleate or atropine, in quantities sufficient to render the tissues insensitive to 5-hydroxytryptamine, histamine or acetylcholine respectively. There were no signs of change in the level of muscular contraction, even after repetitive injection of saliva into the bath.

The effects of the saliva on microcirculation vessel permeability were examined in non-anaesthetized rabbits (Edery & Grunfeld, 1969). Three animals were injected intravenously via the vena marginales, with 60 mg/kg pontamine sky-blue. 15 min later saliva (max. vol. 0·01 ml) was injected into the depilated skin of the back. Animals were killed 30 min after injection and the inner skin was examined. A dark blue spot appeared at the site of injection of saliva, indicating increasing microcircular vessel permeability. This response was slightly weakened by the above antagonists. Spot diameter varied from 15–25 mm; saline (0·1 ml) caused blueing at the needle site only.

The arterial blood pressure of 6 cats was measured with a Statham physiological pressure transducer connected to a Grass polygraph. Injections of 0·1–0·5 ml saliva in the femoral vein produced a transient fall in arterial blood pressure of 25–50 mm/Hg lasting for 10–120 s and a rise in pulse pressure (10–15 mm/Hg). The fall was little depressed by prior injection of antihistamine.

The activity of the saliva did not change after incubation at 37° for 30 min or after boiling for 5 min but it disappeared after dialysis for 24 h at 4° and after incubation with chymotrypsin, papain or pancreatin, but not trypsin. It was not diminished on the ileum in the presence of morphine sulphate (0·03–0·3 mg/ml) suggesting that prostaglandins E_1 and E_2 were not present.

Saliva from larvae of several hornet and wasp species (*Vespa crabro, Paravespula vulgaris, P. germanica, Dolichovespula saxonica, D. media* and *Polistes gallicus*) was examined in the same way. All samples had similar effects on smooth muscle contraction, microcircular vessel permeability, and blood pressure.