Evidence for dopamine receptor stimulation by apomorphine

SIR,—Recently, Ernst (1967) has reported that the apomorphine-induced compulsive gnawing in rats is not mediated via the release of catecholamines, since it is not reduced by the catecholamine synthesis inhibitors α-methyl-3,4-dihydroxyphenylalanine and α-methyltyrosine. On the other hand, the gnawing seen after treatment with (+)-amphetamine is blocked by these synthesis inhibitors. Since the apomorphine-induced gnawing requires an intact corpus striatum and gnawing can also be produced by the catecholamine precursor dihydroxyphenylalanine, Ernst (1967) suggested that apomorphine acts on the dopamine receptors whereas amphetamine acts by releasing dopamine. In the present paper supporting evidence for this view is given by further functional, biochemical and histochemical studies.

The functional influence of apomorphine on dopamine neurotransmission in the corpus striatum was examined after unilateral removal of the corpus striatum of adult hooded rats weighing about 200 g (Andén, Dahlström & others, 1966a). A possible action of apomorphine on the noradrenaline receptors of the spinal cord was tested in acutely spinalized adult hooded rats by evaluating the changes in the flexor reflex evoked by pinching the hind limbs.

The effect of apomorphine on the dopamine and noradrenaline levels of the brain and spinal cord was determined biochemically (Bertler, Carlsson & Rosengren, 1958; Carlsson & Waldeck, 1958) and histochemically (Falck, Hillarp & others, 1962; Dahlström & Fuxe, 1964; Hamberger, Malmfors & Sachs, 1965).

Function. These studies were made mainly on rats which had been pretreated with reserpine (10 mg/kg i.p., 3 hr) plus α-methyltyrosine methylester (H 44/68, 500 mg/kg, i.p., 2 hr) after removal of the left corpus striatum by suction. After this treatment all the operated animals turned towards the unoperated side (cf. Andén & others, 1966a). After injection of apomorphine (1–25 mg/kg s.c.) these rats changed their position and turned or rotated towards the operated side. This effect began about 5 min after the injection and was evident for about 45–60 min. If apomorphine was given to operated rats not pretreated with reserpine–H 44/68 combination, this action of apomorphine, like the gnawing, seemed to be less pronounced. If haloperidol (5 mg/kg i.p.) was given 15–20 min after apomorphine all the rats turned from the operated towards the unoperated side in about 15 min and the gnawing ceased.

(+)-Amphetamine (0·5–25 mg/kg s.c.), like apomorphine, made the rats turn or rotate towards the operated side. In contrast to apomorphine, however, this action of amphetamine was not seen after pretreatment with reserpine plus H 44/68 (cf. Weissman, Koe & Tenen, 1966; Hanson, 1967; Ernst, 1967).

Apomorphine (25 mg/kg s.c.), in contrast to (+)-amphetamine (0·5–25 mg/kg s.c.) and L-3,4-dihydroxyphenylalanine (50–75 mg/kg i.v. 2 hr after nialamide 50 mg/kg i.p.), did not cause a definite increase of the flexor reflex in spinalized rats.

Chemistry. The biochemical results obtained in unoperated adult hooded rats are presented in Table 1. Apomorphine caused a retardation of the depletion in brain dopamine produced by H 44/68. The difference between the dopamine levels in the apomorphine–H 44/68 group and in the H 44/68 group is statistically significant ($P < 0.001$, Student's *t*-test). This action of apomorphine on the brain dopamine was blocked by haloperidol. The disappearance of noradrenaline from the brain and the spinal cord after H 44/68 did not seem to be influenced by apomorphine. (+)-Amphetamine did not cause any significant retardation of the dopamine and noradrenaline loss after H 44/68.

TABLE 1. LEVELS (μG/G; MEAN ± S.E.M.) OF BRAIN DOPAMINE, BRAIN NORADRENALINE AND SPINAL CORD NORADRENALINE IN THE RAT AFTER TREATMENT WITH APOMORPHINE 25 MG/KG S.C., 45 MIN; H 44/68 250 MG/KG I.P., 1 HR; HALOPERIDOL 5 MG/KG I.P., 2 HR; (+)-AMPHETAMINE 25 MG/KG S.C., 45 MIN).

	Dopamine in brain	Noradrenaline in brain	Noradrenaline in spinal cord
No drug treatment	0·80 ± 0·029 (4)*	0·40 ± 0·033 (4)	0·28 ± 0·021 (4)
Apomorphine	0·76 ± 0·034 (4)	0·38 ± 0·022 (4)	0·30 ± 0·019 (4)
H 44/68	0·40 ± 0·015 (11)	0·30 ± 0·012 (11)	0·23 ± 0·012 (11)
H 44/68 + Apomorphine ..	0·55 ± 0·019 (11)	0·28 ± 0·008 (11)	0·21 ± 0·010 (11)
Haloperidol + H 44/68 ..	0·35 ± 0·016 (8)	0·23 ± 0·014 (8)	0·19 ± 0·010 (8)
Haloperidol + H 44/68 + Apomorphine	0·36 ± 0·014 (8)	0·19 ± 0·006 (8)	0·16 ± 0·010 (8)
H 44/68	0·42 ± 0·006 (4)	0·27 ± 0·019 (4)	0·24 ± 0·012 (4)
H 44/68 + Amphetamine ..	0·45 ± 0·015 (4)	0·23 ± 0·018 (4)	0·24 ± 0·017 (4)

* No. of experiments.

In the histochemical studies male Sprague-Dawley rats were used. The rats treated with apomorphine (25 mg/kg s.c. 4½ hr plus 10 mg/kg, s.c. 2 hr before death) plus H 44/68 (250 mg/kg, i.p. 4 hr before death) showed a higher fluorescence intensity in the dopamine terminals of the nucleus caudatus and putamen, nucleus accumbens and the tuberculum olfactorium than did the rats treated with H 44/68 alone. On the other hand, the dopamine terminals of the median eminence, like all the noradrenaline terminals, appeared unaffected by apomorphine. After haloperidol pretreatment (5 mg/kg i.p. 2 hr before death) the dopamine terminals of the rats treated with apomorphine plus H 44/68 seemed to be as depleted as those of the rats treated with H 44/68. As in the biochemical experiments, there was a tendency towards an acceleration of the noradrenaline and dopamine loss after H 44/68 in the haloperidol-treated rats. The reason for using different time-intervals was that in the biochemical experiments it is easier to detect a difference when the amine levels are high, whereas in the histochemical experiments the same is true for low levels.

The apomorphine-induced retardation of the dopamine depletion after H 44/68 is in all likelihood due to reduced activity in the dopamine neurons since a similar retardation after H 44/68 is observed in the noradrenaline nerve terminals lacking an impulse flow such as those in the spinal cord caudal to a transection (Andén, Corrodi & others, 1966b). Such reduced activity in the dopamine neurons can be explained by a negative feed-back mechanism due to a dopamine receptor activation. Such a finding was indicated in the functional studies. The absence of functional and chemical changes by apomorphine after haloperidol treatment may be due to a blockade of the central catecholamine receptors by the latter drug.

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α-Adrenergic blocking action of propranolol

SIR,—The inhibitory action of catecholamines on the rabbit aorta may be observed *in vitro* in strips pretreated with phenoxybenzamine and contracted by adding carbachol to the bathing fluid. When propranolol was used to block this inhibitory action of the catecholamines, the original excitatory action of these compounds was observed by us. This unexpected effect was of interest because similar concentrations of phenoxybenzamine caused complete blockade of the excitatory action of catecholamines in untreated strips. We are investigating the mechanism of this anti-α-adrenergic blocking action of propranolol and have observed that the drug has an α-adrenergic blocking action.

All experiments were done on spirally cut rabbit aortic strips suspended in Krebs-Henseleit solution maintained at 38° bubbled with 5% carbon dioxide in oxygen. Isotonic contractions against 2 g tension and magnified tenfold were recorded on a kymograph.

In these experiments increasing concentrations of propranolol caused an increasing degree of blockade of the excitatory action of noradrenaline. Propranolol at 10^{-6} g/ml produced 0–45%, and at 10^{-5} g/ml produced 30–72% inhibition of noradrenaline (10^{-8} g/ml). Inhibition of 10^{-7} g/ml noradrenaline was also studied; at 3×10^{-5} g/ml, propranolol caused 20% inhibition, while at 10^{-4} g/ml it caused 67–89% inhibition. Complete recovery from the blocking action of a single dose of propranolol occurred in 60–75 min at all levels of testing. The effect of multiple concentrations of noradrenaline in the presence of propranolol was compared with the effect of a unit concentration of noradrenaline without propranolol. At 3×10^{-5} g/ml of propranolol the dose-ratio was between 3 and 5, at 5×10^{-5} g/ml it was between 5 and 10, and at 10^{-4} g/ml of propranolol the dose-ratio was between 30 and 100. The pA_{10} of propranolol (exposure time 5 min) against noradrenaline, derived from seven experiments, was $3{\cdot}7 \pm 0{\cdot}03$ compared with $6{\cdot}2 \pm 0{\cdot}04$ for phentolamine (exposure time 3 min).

These observations suggested that propranolol had an α-adrenergic blocking action. The nature of this action of propranolol was examined further by