DECREASED SPIROPERIDOL AND LSD BINDING IN RAT BRAIN AFTER CONTINUOUS AMPHETAMINE *

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Rats were implanted with a silicone tubing pellet continuously releasing amphetamine base for several days. After five days of this treatment specific binding of spiroperidol (dopamine receptors) and LSD (serotonin receptors) was decreased in the corpus striatum and frontal cortex. In the striatum the number of dopamine receptors was decreased while the affinity was unchanged. These results indicate that dopamine and serotonin receptors can be regulated by the release of their own neurotransmitter.

Continuous amphetamine treatment | Affinity binding | ^{3}H-Spiroperidol | Frontal cortex
Striatum | ^{3}H-LSD | Rat

1. Introduction

It has been shown in controlled studies of amphetamine psychosis in humans that the continuous administration of amphetamine for a number of days produces psychotic symptoms closely resembling schizophrenia (Griffith et al., 1972; Angrist et al., 1974). Furthermore, it has been mentioned that the psychotic behavior thus produced is more closely associated with schizophrenia than is the type of psychotic symptoms induced by a single high dose of the drug (see discussion in Snyder et al., 1974). For this reason we have developed slow-release silicone pellets (Huberman et al., 1977) to achieve a drug regimen similar to that used in the human studies by Griffith and Angrist. Implantation of these pellets in animals produce continuous and intense stereotypies during the first 2-3 days (Ellison et al., 1978). In the later phase (days 4-7) in the rat, spontaneous 'startle' responses, abnormal social behavior and the emergence of heightened grooming, limbflicks, and 'wet-dog' shakes are observed (Ellison et al., 1978; Nielsen et al., 1980). In monkeys there is a similar progression of behavioral changes following implantation of the amphetamine pellet (Lyon and Nielsen, 1979). Here, the late phase is characterized by behaviors suggestive of hallucinations and motor disturbances. An increase in dopamine receptor number has been reported to follow chronic neuroleptic drug treatment of rats (see discussion), and amphetamine psychosis is one of the major bases for the dopamine theory of schizophrenia (Meltzer and Stahl, 1976). For these reasons we thought it of interest to investigate the effect of amphetamine pellet treatment on the receptor systems possibly involved in the psychotomimetic effect of the drug.

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2. Materials and methods

Male Wistar rats weighing between 250-300 g were implanted with a slow-release, silicone tubing pellet containing 50 mg amphetamine base in 200 µl polyethylene glycol. The pellet was constructed so as to release 25 mg of amphetamine base in the 7 days after implantation, producing brain amphetamine concentrations of 1.6 and 0.9 µg/g at 2 and 8 days after implantation, respectively (Huberman et al., 1977). Control rats were implanted with a pellet containing only polyethylene glycol. Rats were decapitated five days after pellet implantation and the corpus striatum (100 mg), the frontal cortex 270 mg), the remaining cortex (occipital plus parietal cortex) (470 mg), hippocampus (100 mg) and the rest of the brain minus cerebellum (300 mg) were homogenized in 0.32 M sucrose. Specific binding of several ligands was measured in a crude mitochondrial/microsomal membrane fraction (P_2) in 50 mM Tris HCl, pH 7.4 by conventional filtration techniques. The total incubation volume was 0.55 ml except when stated otherwise.

^{3}H-Spiroperidol ((1-phenyl-4-^{3}H), 23.6 Ci/mmol, N.E.N., Boston, Mass.) was incubated with 2 mg original tissue for 15 min at 37°C followed by 10 min at 0°C. The method is slightly adapted from that of Leysen et al. (1978a). ADTN (2-amino-6,7-dihydroxy-1,2,3,4,-tetrahydronaphtalene) (10^{-4} M) or spiroperidol (10^{-5} M) were used for obtaining blank values.

^{3}H-LSD (Bennet and Aghajanian, 1975) ((2-^{3}H(N)), 18.3 Ci/mmol, N.E.N., Boston, Mass.) was incubated with 10 mg original tissue for 15 min at 37°C at a concentration of 5 nM. Mianserine (10^{-5} M) was used for obtaining blank values.

^{3}H-Quinuclidinyl benzilate (Yamamura and Snyder, 1974) ((3-^{3}H), 13 Ci/mmol, Amersham, England) was incubated with 3.3 mg original tissue for 1 h at 37°C at a concentration of 1 nM. Atropine (3×10^{-6} M) was used for obtaining blank values.

^{3}H-Naloxone (Squires and Braestrup, 1978) ((^{3}H-G), 17.1 Ci/mmol, N.E.N., Boston, Mass.) was incubated with 10 mg original tissue for 30 min at 0°C at 20 nM. One concentration of nalorphine (3×10^{-7} M) was used to obtain blank values.

^{3}H-Diazepam (Braestrup and Squires, 1977) ((^{3}H-methyl), 14.4 Ci/mmol, gift from W. Haefely, Roche) was incubated with 10 mg original tissue for 30 min at 0°C at a concentration of 1.9 nM. Diazepam (3×10^{-6} M) was used to obtain blank values.

3. Results

Five days after amphetamine-pellet implantation the specific binding of ^{3}H-spiroperidol and of ^{3}H-LSD was reduced in corpus striatum and frontal cortex but not in other regions

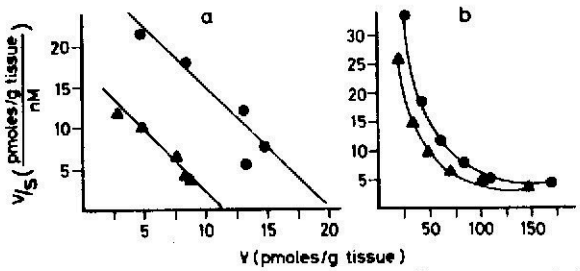

Fig. 1. Scatchard analysis of specific ^{3}H-spiroperidol binding in corpus striatum in placebo pellet-(●——●) and amphetamine pellet- (▲——▲) treated rats. Four independent Scatchard analyses were made for both placebo and amphetamine pellet-treated rats and the values were averaged for each concentration of ^{3}H-spiroperidol. (a) Original tissue, 2 mg, was incubated with ^{3}H-spiroperidol (final concentrations 0.05, 0.1, 0.25, 0.4 and 0.5 nM) in a total volume of 2.5 ml. ADTN (10^{-4} M) was used for blank values. The affinity constant, K_D for placebo and amphetamine pellet treatment was: 0.14 ± 0.02 nM and 0.17 ± 0.04 nM, respectively (mean ± S.E.M. of four values), the total number of binding sites for placebo and amphetamine pellet treatment was 20.0 ± 1.5 pmol/g tissue and 12.7 ± 2.1 pmol/g tissue, respectively (mean ± S.E.M. of four values, $P < 0.05$). (b) Original tissue, 2 mg, was incubated with ^{3}H-spiroperidol (final concentration 1, 2.5, 5, 10, 20 and 40 nM) in a total volume of 0.5 ml. Spiroperidol (10^{-5} M) was used for obtaining blank values.

TABLE 1

Specific ^{3}H-spiroperidol and ^{3}H-LSD binding to brain tissue membranes in rats treated with amphetamine for five days.

Each value is the mean ± S.E.M. of 5 determinations. The whole experiment was repeated twice with similar effects.

^{3}H-QNB, ^{3}H-naloxone and ^{3}H-diazepam binding were measured in an experiment similar to that in the table. ^{3}H-QNB binding of placebo-treated rats: striatum 73.1 ± 1.5 pmol/g tissue (amphetamine pellet 92 ± 1% [4]; frontal cortex 66.1 ± 0.7 pmol/g tissue (amphetamine pellet 95 ± 0.7% [4]; rest of cortex 59.6 ± 0.5 pmol/g tissue (amphetamine pellet 102 ± 1%). ^{3}H-Naloxone binding of placebo-treated rats: striatum 8.5 ± 0.5 pmol/g tissue (amphetamine pellet 95 ± 4%); frontal cortex 9.2 ± 0.1 pmol/g tissue (amphetamine pellet 93 ± 5%); rest of cortex 5.4 ± 0.5 pmol/g tissue (amphetamine pellet 108 ± 5%). ^{3}H-Diazepam binding of placebo-treated rats: striatum 7.1 ± 0.4 pmol/g tissue (amphetamine pellet 108 ± 12% of placebo); frontal cortex 16.3 ± 0.3 pmol/g tissue (amphetamine pellet 95 ± 4% of placebo). Each value is the mean ± S.E.M. of 5 determinations. The protein content of the P_2 pellet from the rats with an amphetamine pellet (50.0 ± 2.0 mg/g tissue; mean ± S.E.M., n = 19, average for all regions) was not different from placebo-treated rats (52.9 ± 1.5 mg/g tissue; mean ± S.E.M., n = 18).

Brain region	Treatment	Specific binding, pmol/g tissue		
		^{3}H-spiroperidol 0.1 nM	^{3}H-spiroperidol 5 nM	^{3}H-LSD 5 nM
Corpus striatum	Placebo [1]	7.50 ± 0.32	52.6 ± 1.3	9.23 ± 0.31
	Amph. pellet [2]	5.50 ± 0.45 [4]	41.7 ± 3.3 [3]	7.24 ± 0.22 [5]
Frontal cortex	Placebo	3.27 ± 0.11	55.6 ± 0.7	8.63 ± 0.78
	Amph. pellet	2.79 ± 0.15 [3]	42.2 ± 2.2 [5]	6.63 ± 0.24 [3]
Rest of cortex	Placebo	2.90 ± 0.07	51.7 ± 1.6	4.76 ± 0.16
	Amph. pellet	2.65 ± 0.09	44.5 ± 2.1 [3]	4.41 ± 0.20
Hippocampus	Placebo	2.30 ± 0.09	66.0 ± 3.5	8.51 ± 0.49
	Amph. pellet	2.39 ± 0.15	64.1 ± 2.2	7.97 ± 0.64
Rest of brain minus cerebellum	Placebo	0.88 ± 0.13	26.5 ± 1.9	6.39 ± 0.35
	Amph. pellet	0.85 ± 0.07	23.5 ± 1.0	5.69 ± 0.11

[1] Placebo rats were implanted with a silicone pellet containing polyethylene glycol.

[2] 'Amphetamine pellet' rats were implanted with a silicone pellet containing 50 mg amphetamine base in polyethylene glycol.

[3] $P < 0.05$, [4] $P < 0.01$, [5] $P < 0.001$ when compared to placebo-treated rats with Student's *t*-test.

(table 1). There was no effect on muscarinic receptors (^{3}H-QNB) except for a marginal though significant decrease in corpus striatum and frontal cortex, nor were there effects on benzodiazepine receptors or on opiate receptors (legend to table 1).

In contrast to the case of striatum and frontal cortex, dopamine is not believed to be a neurotransmitter in parietal cortex, occipital cortex and hippocampus.

The decrease in ^{3}H-spiroperidol and ^{3}H-LSD binding developed slowly. In the acute, intense stereotypy phase 24 h after implantation no effects were found in specific ^{3}H-spiroperidol binding and ^{3}H-LSD binding (data not shown). High concentrations of amphetamine (10^{-5} M) added to the binding assays did not change ^{3}H-spiroperidol or ^{3}H-LSD binding.

Scatchard analysis of ^{3}H-spiroperidol binding in corpus striatum after 5 days of amphetamine intoxication showed that the total number of receptors was decreased for the dopaminergic part of ^{3}H-spiroperidol binding (defined by displacement with ADTN, see below). The non-dopaminergic part of ^{3}H-

spiroperidol binding could not be resolved by straight lines (fig. 1). No changes in affinity constants wre observed.

4. Discussion

The kind of receptors labelled by ^{3}H-spiroperidol and ^{3}H-LSD is not fully known. Pharmacological evidence indicates that ^{3}H-spiroperidol labels dopamine receptors in the corpus striatum and serotonin receptors in the frontal cortex (Leysen et al. 1978a,b; Quik and Iversen, 1979; Howlett and Nahorski, 1978; Fields et al., 1977). This interpretation, however, seems dependent on the concentration of ^{3}H-spiroperidol used since in corpus striatum of rats low concentrations of ^{3}H-spiroperidol (0.05-0.50 nM) can be displaced by the very potent and selective dopamine agonist ADTN (IC_{50} about 10^{-7}; Quik and Iversen, 1979; our unpublished results), while high concentrations of ^{3}H-spiroperidol (5 nM) are not displaced by ADTN ($IC_{50} > 10^{-4}$ M) (unpublished). ^{3}H-Spiroperidol at all concentrations (0.05-40 nM) was displaced to a great extent by spiroperidol and other neuroleptics. Low concentrations of ^{3}H-spiroperidol (0.1 nM) displaceable by ADTN probably label dopaminergic receptors in the striatum while high concentrations label something more, perhaps the "neuroleptic" binding site. ^{3}H-LSD binding in striatum shares some properties with a dopamine/neuroleptic receptor (Burt et al., 1976a) while binding in frontal cortex exhibits characteristics of a serotonin receptor (Bennet and Aghajanian, 1975; Bennet and Snyder, 1975, 1976). We therefore conclude that the continuous amphetamine intoxication reduced the number of dopamine receptors in corpus striatum and that some non-dopamine ('neuroleptic'?) binding sites might also be decreased. The interpretation is less clear for the cortex. These binding sites are probably serotonin receptors, but a dopaminergic/neuroleptic component cannot be excluded.

Adaptive changes in receptors are observed in several conditions (Raff, 1976). Exposure to agonists such as glucagon, insulin, isoproterenol, or bromocryptin decreases the level of respective receptors (Soman and Felig, 1978; Mukherjee et al., 1975; Gavin et al., 1974; Quik and Iversen, 1978). Exposure to antagonists such as haloperidol increases specific ^{3}H-haloperidol binding (Burt et al., 1976b; Muller and Seeman, 1977).

Amphetamine releases dopamine from nerve terminals (Randrup and Munkvad, 1968). This is particularly intriguing because it means that endogenous dopamine, when released at its physiological site, is able to regulate its own receptor. This effect was recently demonstrated by Howlett and Nahorski (1979) and is confirmed by the present results. The physiological consequences of a reduction in dopamine receptors are not clear. Continuous or chronic amphetamine treatment induces tolerance to some pharmacological effects (e.g. anorexia), but other effects, e.g. sterotypy, which is thought to be mainly dopaminergic, may be increased (Klawans and Margolin, 1975; Segal and Mandell, 1974) but decreases have also been reported (Nelson and Ellison, 1978). However it should be considered that stereotypy is affected by several non-dopamine neuron systems and it is not unlikely that the apparent increase in stereotypy is instead a change in the stereotypy profile due to some other cause for example noradrenaline (see Braestrup, 1977).

Amphetamine is believed also to release serotonin from serotonin nerve terminals (Sloviter et al., 1978). The decreased ^{3}H-LSD binding in frontal cortex may therefore be the consequence of increased activation of serotonin receptors. Behaviorally this latter effect is probably manifested by the emergence of wet-dog shakes and limb-flicks as has been reported to follow chronic amphetamine administration in cats and rats (Nielsen et al., 1980; Trulson and Jacobs, 1979).

In conclusion our results show that dopamine receptors and probably serotonin receptors can be regulated by their own neurotransmitter. Furthermore, the observed changes in

receptors were not present during the initial peak stereotypy phase of pellet treatment but developed later in the course of the continuous amphetamine intoxication. We speculate that this can have relevance for the mechanism by which chronic amphetamine treatment produce psychoses in man.

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