clothiapine is more active in the oxotremorine test than would be expected from the results of the *in vitro* tests raises the question as to whether antagonism of oxotremorine-induced tremor depends exclusively on anti-acetylcholine activity, or whether additional factors may be involved.

It is concluded that the ^{3}H-QNB binding assay and the guinea-pig ileum are equally useful for measuring anti-acetylcholine activity *in vitro*. However, neither test permits reliable conclusions to be drawn concerning *in vivo* acetylcholine effects in the brain.

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Noradrenergic influence on the stereotyped behaviour induced by amphetamine, phenethylamine and apomorphine

E. MOGILNICKA*, C. BRAESTRUP**, *Psychopharmacological Research Laboratory, Sct. Hans Mental Hospital, Dept. E, DK-4000 Roskilde, Denmark*

Many experiments indicate that stereotyped behaviour, consisting of sniffing, licking or biting, elucidated by amphetamines and apomorphine, is absolutely dependent on dopamine transmission in the basal ganglia of the mammalian brain (Randrup & Munkvad, 1974, review). Due to the close clinical similarity between the 'amphetamine psychosis' and certain forms of schizophrenia, the detailed study of amphetamine mechanisms and stereotyped behaviour has aroused great interest. It has been suggested that a cholinergic-dopaminergic balance in the brain is important in controlling the intensity of stereotyped behaviour (Arnfred & Randrup, 1968) and this concept has been of value in explaining the modifying influence of cholinergic systems on the primarily dopamine-dependent production of stereotyped behaviour in response to amphetamines. The concept of neuro-transmitter balance has also been of value in analysing the effects of pharmacological agents in some human extrapyramidal disorders, including Parkinsonism (Klawans, 1968).

We do not question the importance of dopamine transmission for stereotyped behaviour or its possible role in the pathogenesis of schizophrenia. We suggest, however, that the qualitative expression of stereotyped behaviour in different behavioural elements, here more specifically as a transition between sniffing, gnawing and licking, is also influenced by central noradrenaline transmission.

All experiments were in male Wistar rats, 200-250 g, housed in individual wire mesh cages (21 × 27 × 16 cm) at 21–23° with free access to food and water except in observation periods when food and water were withdrawn. Each rat was only used once. Apomorphine HCl (0·5 mg kg^{-1}, s.c.), (+)-amphetamine sulphate (5 mg kg^{-1}, s.c.) or β-phenethylamine hydrochloride (40 mg kg^{-1}, s.c.) in saline was injected at the start of the observation period and the rats were observed for the whole period of continuous sniffing induced by these three drugs. The numbers of rats showing occasional or continuous gnawing/biting at the bars (for apomorphine and amphetamine) or gnawing/licking at the bars (phenethylamine) within the stereotypy period, were recorded and included in the Tables. Gnawing is considered as compulsory biting. The whole-brain contents of homovanillic acid and dihydroxyphenyl acetic acid were estimated by the gas chromatographic technique of Braestrup, Andersen & Randrup (1975) and total MOPEG (3-methoxy-4-hydroxyphenylglycol) according to Braestrup (1973).

In our first series of experiments we investigated the ability of drugs with effects on central noradrenaline mechanisms to change the amphetamine or apomorphine-induced stereotyped sniffing into gnawing or biting. The results in Table 1 show that the noradrenaline receptor blocking drug, phenoxybenzamine, the inhibitor of noradrenaline synthesis diethyldithiocarbamate (DDC), which also reduces amphetamine metabolism (Jonsson & Lewander, 1973), and reserpine, which depletes noradrenaline (and dopamine) and markedly reduces its synthesis (Bræstrup & Nielsen, 1975), induce gnawing or biting in apomorphine- or amphetamine-treated rats. Administration of apomorphine or amphetamine alone in these small doses produced only sniffing behaviour and very infrequent licking. The drug clonidine, like phenoxybenzamine and DDC, induced gnawing or biting in amphetamine- or apomorphine-treated rats. Several experiments indicate that clonidine can inhibit central noradrenaline mechanism (Braestrup & Nielsen, 1976), and the lack of antagonism of the clonidine-induced gnawing by phenoxybenzamine (rather an intensification was noted) further supports the classification of clonidine as an inhibitor of at least one population of central noradrenaline neurons, though by a mechanism different

* Present address: Polish Academy of Sciences, Institute of Pharmacology, Ojcowska Str. 52, 31-344 Krakow, Poland.
** Correspondence.

from phenoxybenzamine. The inhibitory effect of clonidine on central noradrenaline mechanisms is more directly supported by the clonidine-induced inhibition of the firing rate of the noradrenaline neurons in the locus coeruleus (Svensson & Trolin, 1975), even when clonidine is administered directly at the locus coeruleus (Svensson, Bunney & Aghajanian, 1975). Clonidine has no dopamine receptor stimulating properties, as judged from its lack of effect on turning behaviour in animals with unilaterally striatal lesions (Voigtlander & Moore, 1973).

Both amphetamine and apomorphine will, when injected alone, induce gnawing or biting in rats at higher dose levels than used in the present study, and one might argue that the observed effects (Table 1) were merely potentiation effects of apomorphine and amphetamine. In a second series of experiments we have obtained evidence that the transfer from sniffing to gnawing or licking is not a potentiation effect. The

Table 1. *Induction of gnawing by apomorphine and amphetamine following pretreatment with drugs modifying noradrenergic function.*

Pretreatment*	Stimulant dopamine drug	Dose mg kg^{-1} s.c.	No. gnawing No. used	% Pos. resp.
Saline	Apomorphine	0·5	0/40	0
Phenoxybenz.	»	0·5	4/5	80
clonidine	»	0·5	5/5	100
Phenoxybenz. + clonidine	»	0·5	5/5	100
DDC	»	0·5	3/5	60
Reserpine†	»	0·5	5/5	100
Reserpine‡	»	0·5	5/5	100
Saline	Amphetamine	5·0	0/30	0
Phenoxybenz.	»	5·0	5/5	100
Clonidine	»	5·0	4/5	80
DDC	»	5·0	3/5	60
Reserpine†	»	5·0	5/5	100

*Phenoxybenzamine, 20 mg kg^{-1}, i.p. (2 h), DDC, 400 mg kg^{-1}, i.p. (2 h) and clonidine, 0·25 mg kg^{-1}, i.p. 30 min before dopamine stimulant drug. Reserpine, 7·5 mg kg^{-1}, s.c.
†24 h or ‡ 3 h before dopamine stimulant drug.

amphetamine-like drug β-phenethylamine induces a very intense sniffing and repetitive head and limb movements in rats, but licking or gnawing is not observed, even after 160 mg kg^{-1} phenethylamine (s.c.) (with 8 mg kg^{-1} of 1-deprenyl injected 5 h before, n = 4). Pretreatment with 1-deprenyl was used in all experiments with phenethylamine to avoid its otherwise fast degradation by MAO-B (Yang & Neff, 1973; Braestrup & others, 1975). After phenethylamine we observed that all the drugs used, including the dopamine β-hydroxylase inhibitor FLA-63, which all inhibit central noradrenaline mechanisms, were able to transfer the phenethylamine-induced sniffing into gnawing or licking (Table 2). It should be emphasized that the transfer to licking or gnawing was evident as soon as 3 h after reserpine when postsynaptic supersensitivity hardly is developed (Dahlström, Fuxe & others, 1967).

The data on phenethylamine thus most clearly suggest that the inhibition of noradrenaline mechanisms induces a qualitative shift in the stereotyped behaviour, and that the effect is not merely a dopamine potentiation as previously believed when sniffing behaviour developed into licking and gnawing. This conclusion is substantiated by biochemical data. Table 3 shows that

Table 2. *Induction of gnawing or licking by phenethylamine following pretreatment with drugs modifying noradrenergic function.*

Pretreatment*	Dose mg kg^{-1}	Time (h) before PEA inj.	No. gnawing or licking No. used	% Pos. resp.
Saline			1/28	3
Reserpine s.c.†	7·5	3	4/5	80
Reserpine s.c.	7·5	20	9/9	100
Phenoxybenz.	20	5	5/9	55
Clonidine	0·5	1	13/15	87
Phenoxybenz. + clondine	20 0·5	2 1	4/4	100
FLA-63	2 × 30	5 and 18	7/10	70

*All groups, except the one receiving reserpine 7·5 mg kg^{-1} s.c. (3 h) before PEA received 8 mg kg^{-1} 1-deprenyl (s.c.). (Knoll, Budapest) 5 h before 40 mg kg^{-1} phenethylamine HCl (s.c.) plus the indicated pretreatment. The group pretreated with saline exhibited intense sniffing, starting 10 min after phenethylamine and lasting 100 min.
†This group received only reserpine and 80 mg kg^{-1} phenethylamine (i.p.).

apomorphine, amphetamine and phenethylamine indeed affect the central dopaminergic systems. Apomorphine decreased the release of dopamine, reflected by a decrease in the level of homovanillic acid, by direct receptor stimulation induced feedback mechanisms (Andén, Rubenson & others, 1967), while amphetamine and phenethylamine increase homovanillic acid, indicating their releasing effect on central dopamine terminals.

Amphetamine, apomorphine and phenethylamine all have some increasing effect on the level of the major methylated noradrenaline metabolite in the cns, MOPEG, indicating that these drugs induce a release of noradrenaline in the cns. The release induced by phenethylamine in the presence of 1-deprenyl, however, appears to be very high and this high release of noradrenaline probably inhibits the development of licking and gnawing by phenethylamine. This view is further supported by the result that the gnawing or licking induced by a high dose of apomorphine (2 mg kg^{-1} s.c., n = 8) were completely inhibited by phenethylamine (80 mg kg^{-1}, i.p., n = 8), sniffing was still present with high intensity.

Taken together, these findings strongly indicate that the expression of dopaminergic induced stereotyped behaviour is dependent on the degree of noradrenergic transmission in the cns. A modulatory but unspecified

Table 3. *The major catecholamine metabolites, homovanillic acid (HVA), 3,4-dihydroxyphenyl acetic acid (DOPAC) and total 3-methoxy-4-hydroxyphenyl-glycol (MOPEG) in the whole rat brain after treatment with apomorphine, (+)-amphetamine sulphate or phenethylamine HCl.*

Treatment	Dose mg kg^{-1}	Time before death	Brain concn (% controls§) means ± s.e.m. HVA	DOPAC	MOPEG
Apomorphine	0·5	1	64·4 ± 3** (n = 6)	73 ± 3** (n = 6)	121 ± 7* (n = 6)
Amphetamine	10	2	183 ± 6** (n = 4)	62 ± 6** (n = 5)	154 ± 3** (n = 4)
Phenethylamine	40†	2	152 ± 6** (n = 5)	90 ± 1** (n = 5)	218 ± 13** (n = 5)

*P<0.05 **P<0.01 Student's *t*-test of treated group versus vehicle treated group, analysed on the same day.
§Control concentrations in whole rat brain; HVA65.7±2.5 ng g^{-1} (11); DOPAC 96 ± 4 ng g^{-1} (11); total MOPEG 72.8 ± 2 ng g^{-1} (15) all corrected for recovery.
†This group was treated with 2 mg kg^{-1}, s.c. of 1-deprenyl 5 h before phenethylamine.

role for noradrenaline on dopaminergically mediated behaviour was also suggested by others (Randrup, Munkvad & Udsen, 1963; Ungerstedt, 1971), while no indication has yet been presented that lesions in the noradrenaline neurons by 6-hydroxydopamine influence stereotyped behaviour (Creese & Iversen, 1975; Roberts, Zis & Fibiger, 1975). The noradrenergic influence on the transfer from drug-induced sniffing behaviour to licking or gnawing behaviour reported in the present study indicates that noradrenaline influences should be considered in behavioural studies on drugs stimulating the dopamine system. Further, the conclusion of the present study implicates that central noradrenergic mechanisms should be regarded together with dopamine when the amphetamine model for schizophrenia is used for screening of new antipsychotic drugs or for studies of the pathogenesis of the disease.

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