Effects of (±)-10-hydroxy-*N*-n-propylnoraporphine on catecholamine turnover in the rat brain

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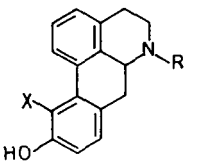
Monohydroxy aporphines substituted in either the 10- or 11-position stimulate turning away from the side of the lesion in animals with unilateral lesions of the striatum produced by the intracerebral injection of 6-hydroxydopamine (Neumeyer, Granchelli & others, 1974; Neumeyer, Dafeldecker & others, 1976) and produce stereotyped behaviour after systemic injection in rats suggesting that they are dopamine agonists. 10-Hydroxy-*N*-n-propylnoraporphine (10-OH-PNA) was ineffective in stimulating adenyl cyclase from rat striatum (Miller, Kelly & Neumeyer, 1976) and since this enzyme is thought to be the dopamine receptor, this observation suggested that 10-OH-PNA may not act directly on dopamine receptors. The effect of apomorphine on dopamine turnover is thought to result from the stimulation of dopamine receptors (Andén, Rubenson & others, 1967). We have therefore investigated whether 10-OH-PNA can decrease dopamine turnover in the rat striatum as measured by the disappearance of dopamine after α-methyl-*p*-tyrosine (α-MT). The effect of α-MT on the intensity of stereotyped behaviour produced by 10-OH-PNA was also assessed to determine whether this effect of 10-OH-PNA was due to the release of newly synthesized dopamine.



R	X	
CH_3	OH	Apomorphine
C_3H_7	OH	*N*-n-Propyl-nor-apomorphine
C_3H_7	H	10-Hydroxy-*N*-n-propyl norapomorphine

Catecholamine turnover in striatum. Male, albino rats (Sprague-Dawley, Charles River Farms) 130–200 g were used. (±)-10-OH-PNA suspended in 0·5% carboxymethylcellulose (vehicle), was administered intraperitoneally in a dose equivalent to 15 mg kg^{-1} (Schoenfeld, Neumeyer & others, 1975) in 1 ml kg^{-1} body weight. α-Methyl-*p*-tyrosine methyl ester, (Sigma) was dissolved in normal saline and injected intraperitoneally in a volume of 1 ml kg^{-1} at a dose of 200 mg kg^{-1}.

Four groups of eight rats were used. Group I received vehicle; Group II received 10-OH-PNA; Group III α-MT; Group IV received α-MT and 10-OH-PNA. The rats were injected with either 10-OH-PNA or vehicle and 15 min later α-MT or saline. At 90 min, a second injection of 10-OH-PNA or vehicle was given, and the rats were decapitated at 135 min (120 min after the injection of α-MT or saline). The brains were removed and the striatum dissected according to Glowinski & Iversen (1966), weighed and homogenized in 10 volumes of 0·4 N perchloric acid. After centrifugation (30 000 *g* for 20 min), noradrenaline and dopamine were adsorbed on alumina by a modification of the procedure of Black, Hendry & Iversen (1971). Briefly, after adding 1 ml of 4% ethylenedinitrilo tetraacetic acid disodium salt, the supernatant was brought to pH 8·5 with 3 M tris buffer and passed over a column containing 500 mg of alumina which was washed successively with 15 ml of 0·005 M tris and 5 ml water. Dopamine and noradrenaline were eluted with 3 ml of 0·2 M acetic acid and 1 ml of eluate was treated according to Chang (1964). Statistical differences were analysed by Student's *t*-test (one tail).

Stereotyped behaviour. Male albino rats (120–150 g) were injected with drugs or vehicle, and the intensity of behaviour was assessed for each minute of 1 h according to the scale of Ernst (1967) and Butterworth, Poignant & Barbeau (1975), the mean value for each rat for each 5 min period being used in the assessment. Statistical differences in behaviour were determined using the Mann-Whitney U-test, a non-parametric test (Goldstein, 1965).

Catecholamine turnover. 10-OH-PNA did not significantly alter dopamine and noradrenaline concentrations in the striatum while α-MT produced approximately a 51 and 44% decrease respectively. Although 10-OH-PNA with α-MT did not significantly affect the depletion

Table 1. *Effects of 10-OH-PNA on catecholamine turnover in the striatum.* The concentration of noradrenaline and dopamine were determined in the striatum of rats treated as described in the text. Results are the mean ± s.e.m. of 8 determinations.

Treatment	Noradrenaline $\mu g\ g^{-1}$	Dopamine $\mu g\ g^{-1}$
Vehicle	0·797 ± 0·066	7·249 ± 0·435
10-OH-PNA	0·869 ± 0·059	8·368 ± 0·472
α-MT	0·445 ± 0·059	3·573 ± 0·575
10-OH-PNA + α-MT	0·496 ± 0·046	4·977 ± 0·436[a]

[a] Differs from α-MT-treated group, $P > 0·05$.

* Correspondence.

of noradrenaline caused by α-MT alone, it significantly retarded the depletion of dopamine caused by α-MT.

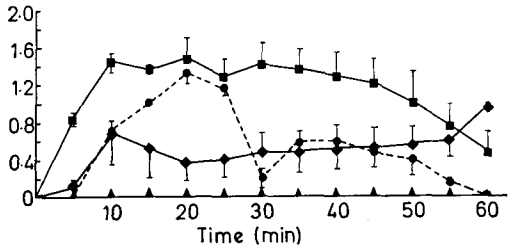


FIG. 1. Effect of α-MT on stereotyped behaviour produced by 10-OH-PNA. Rats were injected with α-MT or saline 1·5 h before receiving 10-OH-PNA or vehicle (carboxymethylcellulose—0·5% solution). The animals were observed for stereotyped behaviour at 1 min intervals for 1 h. For purposes of comparison, some rats were injected with *N*-n-propylapomorphine (1 mg kg^{-1}), the catechol derivative of 10-OH-PNA and a potent stimulant of stereotyped behaviour. The results are expressed as mean score of each animal per min for 5 min ± s.e.m. and represent 5 determinations for each group. Ordinate—Intensity of stereotyped behaviour (Ernst scale). ■—*N*-n-propylnorapomorphine (1 mg kg^{-1}). ●—10-OH-PNA-*N*-n-propylnorapomorphine (15 mg kg^{-1}) after α-MT (200 mg kg^{-1}). ◆—10-OH-*N*-n-propylnorarpophine (15 mg kg^{-1}). ▲—Vehicle treated or α-MT (200 mg kg^{-1}) injected alone.

Stereotyped behaviour. The dose of 10-OH-PNA (15 mg kg^{-1}) used is slightly higher than the ED50 for producing stereotyped behaviour (11·0 mg kg^{-1}) reported by Schoenfeld & others (1975). Vehicle-treated rats showed no stereotyped behaviour at any time over the 60 min period. At all times after 5 min, at least 3 out of 5 rats treated with 10-OH-PNA showed stereotypy but always of low intensity.

α-MT in rats given 10-OH-PNA caused an increase in the intensity of stereotyped behaviour *significantly* greater than that of the group treated with 10-OH-PNA alone at 20 and 25 min after injection.

Thus 10-OH-PNA resembles the effects of apomorphine on stereotyped behaviour and dopamine turnover. In a dose that produces stereotyped behaviour in rats, it decreases dopamine but not noradrenaline turnover in the striatum. It is, therefore active in two of three systems used to evaluate dopaminergic activity. In rats unilaterally lesioned with 6-hydroxydopamine, it induced rotation away from the side of the lesion, (Neumeyer & others, 1974, 1976) and it induces stereotyped behaviour (Schoenfeld & others, 1975).

However, it was ineffective in stimulating adenylate cyclase activity in homogenates of rat striatum under conditions which both dopamine and apomorphine are effective (Miller & others, 1976).

One possible explanation for the differential effects of 10-OH-PNA on behaviour and dopamine-stimulated adenyl cyclase activity is that the compound may not act directly on dopamine receptors but indirectly by releasing dopamine into the synaptic cleft. Compounds like amphetamine that stimulate dopamine receptors by releasing dopamine, do not stimulate adenyl cyclase activity in homogenates (Miller, Horn & others, 1974). If 10-OH-PNA produces stereotyped behaviour by releasing dopamine, pretreatment with α-MT would be expected to inhibit this effect, but it did not, suggesting that 10-OH-PNA produces stereotyped behaviour by the direct stimulation of dopamine receptors. However, the possibility that 10-OH-PNA may release granular stores of dopamine, similar to methylphenidate (Scheel-Kruger, 1971) cannot be excluded nor can the possibility that 10-OH-PNA is converted *in vivo* into the catechol analogue of apomorphine, *N*-propylnorapomorphine. Indeed catechol analogues of apomorphine are much more potent in producing stereotyped behaviour than the monohydroxy analogue (Schoenfeld & others, 1975). The failure of α-MT pretreatment to block the stereotyped behaviour produced by 10-OH-PNA suggests that tyrosine hydroxylase is not the enzyme responsible for converting the monohydroxy derivative to the dihydroxylated form (if such a change does occur).

Pretreatment of rats with α-MT lowered striatal noradrenaline and dopamine to concentrations significantly below the baseline. 10-OH-PNA retarded the disappearance from the striatum of dopamine but not noradrenaline produced by α-MT. Thus the effect of 10-OH-PNA appears selective for dopamine containing neurons.

It had originally appeared that a catechol moiety would be essential for the stimulation of dopamine receptors in the striatum (Costall, Naylor & Pinder, 1974). However, the present study, in agreement with Schoenfeld & others (1975) suggests it is not a necessary requirement.

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Behavioural and biochemical effects of substance P injected into the substantia nigra of the rat

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Increasing biochemical and immunocytochemical evidence suggests that the undecapeptide substance P (SP) may function as a neurotransmitter in the mammalian cns (Konishi & Otsuka, 1975; Cuello, Polak & Pearse, 1976). In the rat there is strong evidence for pathways utilizing SP as a transmitter descending from the caudate nucleus and globus pallidus to the substantia nigra, where SP is found in the highest concentrations in the zona reticulata (Kanazawa & Jessell, 1976; Kanazawa, Emson & Cuello, 1977). Depolarizing stimuli have been shown to release SP from slices of SN tissue in a calcium-dependent fashion (Jessell, 1977), whilst Davies & Dray (1976) found that microiontophoretically-applied SP excited nigral neurons. It is possible, therefore, that the peptide from this striatonigral pathway normally acts to control the firing rate of the reciprocal ascending nigrostriatal dopaminergic neurons. To test this suggestion pure synthetic SP was injected unilaterally into the rat's substantia nigra and the animals observed subsequently for evidence of rotational locomotor activity characteristic of nigrostriatal activation (Ungerstedt, 1971). The concentrations of dopamine and its major metabolite homovanillic acid (HVA) present in the corresponding corpora striata were determined as a biochemical measure of dopaminergic cell activity.

Male Wistar albino rats (Tuck), 200–250 g, were lightly anaesthetized with a mixture of oxygen, nitrous oxide and halothane. One ng of SP was injected stereotactically in a volume of 0·1 μl into the right substantia nigra using a 1 μl Hamilton syringe. Control animals received an equivalent volume of physiological saline. On recovery, animals were placed in a rectangular box and observed for rotational and other stereotyped behaviour. Ten min after injection the rats were stunned and decapitated and the brains quickly removed and frozen in a solid CO_2/methanol mixture. The corpora striata from both hemispheres were subsequently dissected out according to the method of Glowinski & Iversen (1966) and assayed fluorimetrically for dopamine (Shellenberger & Gordon, 1971) and HVA (Murphy, Robinson & Sharman, 1969). The sites of all injections were determined histologically.

As little as 1 ng SP was sufficient to induce circling movement in rats. Table 1 shows that the direction of this turning response was dependent on the site of injection and correlated with nigrostriatal dopamine cell activity. Injections into the zona reticulata invariably elicited contralateral turning which reached a peak frequency of approximately 4 rotations min^{-1} at 7 min after injection, but which faded by 10 min. The short duration may have been caused by breakdown of the SP by peptidases. Locomotion was typically slow and intermittent and was frequently

Table 1. *Direction of rotation and changes in striatal HVA concentrations induced by SP in the rat.*

Dose of SP (ng)	n	Injection site	Rotation	Mean striatal HVA levels (μg g^{-1} wet wt) Left	Right	Diff. (%)
1	9	Zona reticulata	Contra-lateral	0.409	0.660	+36.9*
1	7	Anterior or dorsal to zona recticulata	Ipsilateral	0.695	0.551	−20.7*
0	7	All areas Injection	None	0.503	0.479	− 4.7

All injections were given in a volume of 0.1 μl.
S.e.m. all in the range ±6–10%.
* $P < 0.001$ by paired t-test.

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