Drug-induced changes of dopamine turnover in striatum and limbic system of the rat

Neuroleptic and cholinomimetic compounds increase the cerebral homovanillic acid (HVA) content indicating an enhanced dopamine turnover (Andén, Roos & Werdinius, 1964; Laverty & Sharman, 1965; Perez-Cruet, Gessa & others, 1971); this effect is antagonized by anti-acetylcholine agents (O'Keeffe, Sharman & Vogt, 1970; Perez-Cruet & others, 1971). However, in the rabbit the dopaminergic neurons of the striatum and the limbic system show differences in their response to these substances (Andén & Stock, 1973; Andén, 1974) for reasons not yet fully understood. Therefore, the present work deals with another species, i.e. the rat, in which the changes in limbic and striatal HVA have been compared after various neuroleptics and a cholinomimetic agent, alone or in combination with an antimuscarinic drug.

Male albino rats (strain Füllinsdorf, SPF bred), 150–180 g, were injected with chlorpromazine HCl (5 mg kg^{-1}), haloperidol (0·1 mg kg^{-1}) or clozapine (8-chloro-11-(4-methyl-1-piperazinyl)-5H-dibenzo<b,e> <1,4>diazepine, 20 mg kg^{-1}) i.p. either alone or 30 min after administration of trihexyphenidyl (Artane, 50 mg kg^{-1}) i.p. The animals were decapitated 2 h later. Other rats were injected with oxotremorine (1 mg kg^{-1}, i.p.) 15 min after administration of methylatropine nitrate (10 mg kg^{-1})i.p. alone or combined with various doses of trihexyphenidyl. The animals were killed 1 h after administration of oxotremorine. The lateral ventricles were opened and the corpora striata dissected. Furthermore, a frontal section was performed through the optic chiasma. The part of the brain rostral to this section contains the limbic areas rich in dopamine, referred to as limbic system. HVA was determined in the pooled corpora striata or limbic systems from two rats by extraction with n-butylacetate (Murphy, Robinson & Sharman, 1969) and spectrofluorimetric measurement (Andén, Roos & Werdinius, 1963). Rats injected with 0·9% NaCl served as controls. Drug-induced hypothermia was prevented by keeping the animals at an ambient temperature of 32°. Statistical significance was determined according to Student's *t*-test after a one or two way analysis of variance.

Table 1 demonstrates that the neuroleptic drugs caused an average percentage increase of HVA which was higher in the striatum than in the limbic system. The difference in response between the two brain areas reached statistical significance

Table 1. *Effect of neuroleptics alone or in combination with trihexyphenidyl on the homovanillic acid (HVA) content in the striatum and limbic system of rats.* The results are averages with s.e.m. of 5–7 experiments. Absolute control values (= 100%) of HVA in ng g^{-1}: striatum 820 ± 27, limbic system 208 ± 9.

	HVA Increase (% of controls)			
	Neuroleptic alone		Trihexyphenidyl* + neuroleptic	
Neuroleptic (mg kg^{-1}, i.p.)	Striatum	Limbic system	Striatum	Limbic system
Chlorpromazine (5)	328±18[a]	210±9	213±23[b]	155±17[b]
Haloperidol (0·1)	237±5[a]	174±16	204±12[c]	168±6
Clozapine (20)	250±16	213±18	171±13[b]	165±14[c]

*50 mg kg^{-1}, i.p. 30 min before neuroleptics.
a, $P<0.01$ versus limbic system.
b, $P<0.01$ versus neuroleptic alone.
c, $P\simeq 0.05$ versus neuroleptic alone.

after chlorpromazine and haloperidol. Similar results were obtained with various doses of the three neuroleptics in rats in which drug-induced hypothermia was not prevented (Pletscher, 1975). These findings are in partial disagreement with those obtained in rabbits showing that clozapine caused a less marked HVA rise in the striatum than in the limbic system and that haloperidol increased the HVA content in both regions to about the same extent (Andén & Stock, 1973). However, the possibility cannot be excluded that this difference between rat and rabbit is connected with different transport mechanisms for HVA. Table 1 also shows that the anti-acetylcholine agent trihexyphenidyl counteracted the HVA increase due to the neuroleptics. In agreement with previous results (Andén, 1972) this antagonism seemed to be more marked in the striatum than in the limbic system.

In contrast to the neuroleptic drugs, oxotremorine, a cholinomimetic agent, induced in the rat a more marked HVA elevation in the limbic system than in the striatum (Fig. 1), whereas no major differences between the two regions were observed in the rabbit (Andén, 1974). Trihexyphenidyl counteracted the oxotremorine-induced HVA increase in both the striatum and the limbic system of rats (Fig. 1 and Andén, 1974). The dose of trihexyphenidyl which completely prevented the effect of oxotremorine in the striatum was lower than that required for an equal effect in the limbic system; higher doses of trihexyphenidyl even reduced the striatal HVA content below control values. The antiacetylcholine drug by itself did not decrease the striatal and limbic HVA content (results not shown).

The response to drugs of striatal and limbic dopamine neurons may, as previously

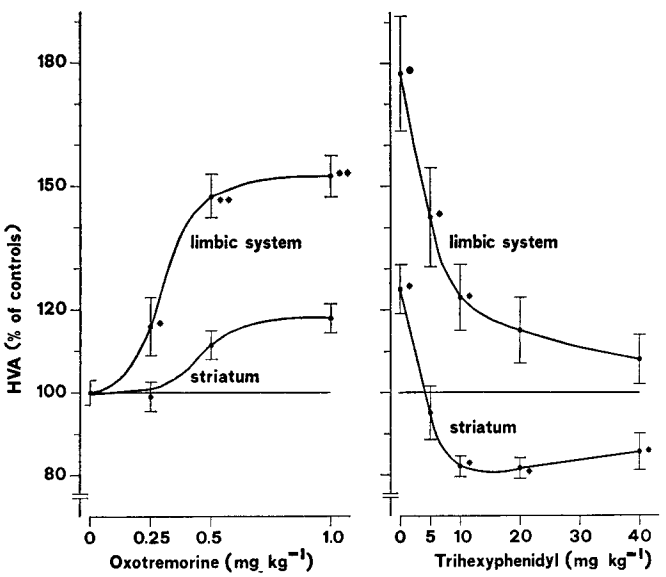

FIG. 1. Left: Effect of various doses of oxotremorine (abscissa) on the homovanillic acid (HVA) content in the striatum and limbic system of the rat.
Right: Effect of various doses of trihexyphenidyl on the HVA rise induced by 1·0 mg kg^{-1} oxotremorine. Oxotremorine was administered 15 min after injection of methylatropine nitrate (10 mg kg^{-1}) alone (left) or combined with trihexyphenidyl (right); the rats were killed 1 h later. All drugs were given intraperitoneally. Each point represents the mean with s.e.m. of 9–18 single values, each determined in the pooled corpora striata or limbic systems of two rats. The HVA values are expressed as % of saline-treated controls (=100%; absolute control values see Table 1). The zero point on the abscissa (right) indicates the HVA increase after oxotremorine alone.
Significance: left: * $P<0{\cdot}05$, ** $P<0{\cdot}01$ versus corresponding values in striatum. Differences from controls: All values with 0·5 and 1·0 mg kg^{-1} oxotremorine: $P<0{\cdot}01$; value with 0·25 mg kg^{-1} oxotremorine in limbic system: $P<0{\cdot}05$.
right: * $P<0{\cdot}01$ versus corresponding controls (100%).

suggested (Andén, 1974), be connected with differences in receptor sensitivity. The pre- and/or post-synaptic dopamine receptors in the striatum are possibly more sensitive to the blocking action of neuroleptics than those in the limbic system. Alternatively, acetylcholine receptors in the striatum might be less sensitive to cholinomimetic compounds than those in the limbic system. However, it has to be considered that the activity of the limbic and striatal dopaminergic system may be regulated not only by common mechanisms [e.g. via the presynaptic dopamine receptors (Carlsson, 1974)], but also by different neuronal inputs determining the response to drugs. In fact, a cholinergic influence is thought to activate the nigrostriatal dopaminergic pathway (Laverty & Sharman, 1965; Corrodi, Fuxe & others, 1967; Perez-Cruet & others, 1971) which in turn inhibits cholinergic neurons in the striatum (Bartholini, Stadler & Lloyd, 1973). In the limbic system too, the dopamine neurons are stimulated by a cholinergic influence (Andén, 1974), but do not seem to modify cholinergic activity (Lloyd, Stadler & Bartholini, 1973). According to this model, the primary activation of dopamine turnover due to stimulation of cholinoceptive sites by oxotremorine may decrease an endogenous excitatory cholinergic input to dopamine neurons in the extrapyramidal but not in the limbic system. This would result in a less marked activation of dopamine turnover in the striatum than in the latter structure. This hypothesis might explain also why the HVA level increased by oxotremorine was more markedly diminished in the striatum by trihexyphenidyl (and even reduced below control levels) than in the limbic system.

Based on this view, it might furthermore be explained why the striatum was more sensitive than the limbic system with regard to (a) the neuroleptic-induced HVA rise and (b) the antagonism by trihexyphenidyl of the neuroleptic-induced increase of this acid. Thus, the activation of the limbic dopamine turnover possibly results from the blockade of presynaptic dopamine receptors, whereas in the striatum, in addition to this mechanism, the dopamine turnover may be increased by a cholinergic input activated as a result of blockade of postsynaptic dopamine receptors (Bartholini & others, 1973).

In conclusion, the different response of the striatum and the limbic system to neuroleptic and antiacetylcholine drugs may be related to differences of receptor sensitivity and/or of dopaminergic influences on the cholinergic neurons, whereby species differences have to be taken into consideration.

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Effect of metoclopramide on turnover of brain dopamine noradrenaline and 5-hydroxytryptamine*

Metoclopramide (4-amino-5-chloro-*N*-[2-(diethylamino) ethyl]-*O*-anisamide) a powerful anti-emetic agent, has been used in various clinical conditions, including Parkinson's disease. However, in a small percentage of patients it causes acute dystonic reactions similar to those produced by neuroleptic drugs such as phenothiazines and butyrophenones (Borenstein & Bles, 1965; Casteels-Van Daele, Jaeken & others, 1970; Robinson, 1973). The most characteristic biochemical effect of neuroleptics is their capacity to increase central dopamine turnover (Carlsson & Lindqvist, 1963; Andén, Butcher & others, 1970; Nybäck & Sedvall, 1969) due, it is believed, to blockade of dopamine receptors causing an increase in firing of dopaminergic neurons (Bunney, Walters & others, 1973). In the present investigation we have studied the effect of metoclopramide on dopamine turnover by measuring dopamine and its principal metabolite homovanillic acid (HVA) in whole brain, and in corpus striatum and the mesolimbic area both of which contain large quantities of dopamine (Andén, 1972; Lloyd, Stadler & Bartholini, 1973). In addition, we have examined the effect of metoclopramide on whole brain levels of noradrenaline and its principal metabolite, 4-hydroxy-3-methoxy-phenylglycol sulphate (MOPEG-SO_4), and on 5-hydroxytryptamine (5-HT) and its principal metabolite, 5-hydroxyindoleacetic acid (5-HIAA).

Metoclopramide was injected intraperitoneally into Swiss "S" strain mice (approximately 30 g). The whole brains were quickly removed and deep-frozen. Brain parts were dissected on an ice-cold Petri-dish and then deep-frozen immediately. A transverse cut was made behind the striata, which were excised, and the cortex and the adhering part of the hypothalamus were removed. The forebrain tissue remaining contained the corpora amygdala, the olfactory tubercle and the nucleus accumbens (mesolimbic area). Parts from 3 animals were pooled for each determination. Dopamine was estimated by the method of Chang (1964), HVA by the method of Murphy, Robinson & Sharman (1969), noradrenaline by the method of Maickel, Cox & others (1968) and 5-HT and 5-HIAA by the method of Curzon & Green (1970). MOPEG-SO_4 was estimated in male Wistar rats according to Meek & Neff (1972) as this metabolite does not seem to be a major degradation product of noradrenaline in the mouse (Ceasar, Hague & others, 1974).

The results are summarized in Fig 1 and Table 1. Metoclopramide had no effect on whole brain dopamine concentrations, but caused a dose-dependent increase in whole brain HVA, which was maximal 1·5 h after injection. Metoclopramide increased HVA concentrations both in the corpus striatum and in the mesolimbic area, to approximately the same extent (i.e. by a factor of 4·5 and 5·9 respectively). Metoclopramide had no significant effect on whole brain noradrenaline, MOPEG-SO_4, 5-HT or 5-HIAA concentrations.

* Since this study was completed, Ahtee & Buncombe (*Acta pharm. tox.*, 1974, 35, 429-432) also have shown that metoclopramide causes a dose-dependent increase in mouse striatal HVA correlated with the intensity of catalepsy produced, and they suggest that metoclopramide blocks striatal dopaminergic receptors.