SNYDER, S. H. & COYLE, J. T. (1969). *J. Pharmac. exp. Ther.*, **165**, 78–86.
THIERRY, A. M., STINUS, L., BLANC, G. & GLOWINSKI, J. (1973). *Brain Res.*, **50**, 230–234.
UNGERSTEDT, U. (1971). *Acta physiol. scand., Suppl.*, **367**, 1–122.
WALDECK, B. (1968). *J. Pharm. Pharmac.*, **20**, 111–115.

Antagonistic effects of apomorphine and haloperidol on rat striatal synaptosomal tyrosine hydroxylase

Haloperidol and several other antipsychotic neuroleptics have been shown to accelerate the turnover of dopamine in brain (Carlsson & Lindqvist, 1963; Andén, Corrodi & Fuxe, 1972; Andén, Roos & Werdinius, 1964; Cheramy, Besson & Glowinski, 1970) as well as to accelerate the rate of firing of dopamine neurons (Bunney, Walters & others, 1973). There is now good evidence that this effect of neuroleptics is a consequence of both pre- and post-synaptic (Kehr, Carlsson & others, 1972; Bunney & Aghajanian, 1973) dopamine receptor blockade, although the exact mechanisms involved remain to be elucidated. Apomorphine, a substance which appears to directly stimulate dopamine receptors, exerts effects on dopamine turnover opposite to those produced by neuroleptics: it retards the turnover of dopamine, which has also been measured in several ways (Roos, 1969; Nybäck, Schubert & Sedvall, 1970; Goldstein, Freedman & Backstrom, 1970), inhibits the activity of dopamine neurons (Bunney, Aghajanian & Roth, 1973) and antagonizes the effects of neuroleptics (Andén, Rubenson & others, 1967; Lahti, McAllister & Wozniak, 1972; Kehr & others, 1972).

The interruption of impulse flow in dopamine neurons, either by axotomy or by the administration of γ-hydroxybutyric acid, causes a 60 to 100% increase in striatal dopamine (Walters, Roth & Aghajanian, 1973) an effect that can be blocked by apomorphine (Andén, Magnusson & Stock, 1973). Furthermore, the *in vivo* inhibition of tyrosine hydroxylase by apomorphine in denervated forebrain was shown to be partially reversed by the simultaneous administration of haloperidol (Kehr & others, 1972). These findings strongly suggest that dopamine neurons are, at least partly, autoregulated; released or extraneuronal dopamine seems to act on presynaptic, inhibitory dopamine receptors. Stimulation of the inhibitory dopamine receptors on dopamine neurons, either by dopamine or apomorphine, may inhibit both nerve activity and dopamine synthesis.

Striatal synaptosomes have relatively high tyrosine hydroxylase activity (McGeer, Bagchi & McGeer, 1965; Cicero, Sharpe & others, 1972), which is not dependent on added cofactors in contrast to non-synaptosomal tyrosine hydroxylase (Karobath, 1971). Since synaptosomes may function as a pure *in vitro* pre-synaptic system, with little or no influence by post-synaptic events, we decided to test the effects of apomorphine and haloperidol on tyrosine hydroxylase activity in striatal synaptosomes.

Tyrosine hydroxylase activity was measured both as $^3H\text{-}H_2O$ formed according to Nagatsu, Levitt & Udenfriend (1964) and accumulated [^{3}H]catecholamines according to the slightly modified procedure of Renzini, Brunori & Valori (1970) from L[^{3}H]3,5-tyrosine (70 Ci mmol^{-1}), which was purified on a Dowex-50 column from which it was eluted with N ammonium hydroxide. The eluate was stored at 4° for up to 14 days. Just before use a suitable volume of the tyrosine eluate was evaporated to dryness at room temperature (20°) under a stream of nitrogen, and taken up in oxygen-saturated Krebs-Ringer phosphate containing glucose, ascorbate and EDTA as described by Kuhar, Roth & Aghajanian (1972) to give about 2 million counts min^{-1} per assay.

Table 1. *Effects of apomorphine and haloperidol on tyrosine hydroxylase activity in striatal synaptosomes.* All results are the mean values ± s.d. of 2 determinations.

Apomorphine concentration in incubation medium (M)	Haloperidol concentration in incubation medium (M)	Total counts min^{-1} minus blank value[b] in ^{3}H-CA assay	% reversal	Total counts min^{-1} minus blank value[c] in ^3H-H_2O assay	% reversal
0	0	32 488 ± 1965		67 515 ± 3152	
$6{\cdot}5 \times 10^{-7}$	0	19 402 ± 223		33 666 ± 240	
0	$1{\cdot}1 \times 10^{-7}$	33 296 ± 1303		69 867 ± 1382	
0	$2{\cdot}2 \times 10^{-7}$	31 823 ± 1847		68 065 ± 670	
0	$4{\cdot}4 \times 10^{-7}$	28 960 ± 1878		65 777 ± 3755	
$6{\cdot}5 \times 10^{-7}$	$1{\cdot}1 \times 10^{-7}$	26 521 ± 293[a]	51	54 106 ± 3169[a]	56
$6{\cdot}5 \times 10^{-7}$	$2{\cdot}2 \times 10^{-7}$	26 496 ± 1195[a]	57	52 392 ± 1340[a]	54
$6{\cdot}5 \times 10^{-7}$	$4{\cdot}4 \times 10^{-7}$	24 113 ± 757[a]	49	54 258 ± 124[a]	64

(a) *P*-values ranged from <0·001 to 0·015 compared with the apomorphine-group (Student's *t*-test).
(b) The blank value in [^{3}H]catecholamine (^{3}H-CA) assay was 300 counts min^{-1}.
(c) The blank value in ^3H-H_2O assay was 20 300 counts min^{-1}.

Less than 20 min before the start of the experiment, corpora striata were rapidly excised from rat brains immediately after death, homogenized in 10 volumes of ice cold 0·32 M sucrose with a Potter-Elvehjem glass homogenizer and Teflon pestle (150 μm clearance). Incubations were carried out in a total volume of 750 μl containing 50 μl striatal homogenate, 100 μl test substances and 600 μl Krebs-Ringer-phosphate with [^{3}H]3,5-tyrosine mixed at 0°, transferred to a 37° bath for 60 min and returned to the ice-bath. Perchloric acid (25 μl 70%) was added to precipitate protein and unlabelled dopamine-carrier (8 μg) added to protect newly formed [^{3}H]catecholamine. After centrifugation the supernatant was adjusted to pH 6·5 with 0·5 M Na_3PO_4 and passed through an Amberlite CG-50 column (25 × 5 mm, buffered to pH 6·5). The Amberlite column was then washed twice with 2·0 ml water. The second 2·0 ml was discarded while the first 2·0 ml was combined with the effluent from the supernatant and transferred to a Dowex-50 column (25 × 5 mm, H^+-form) after adjusting pH to about 2 with HCl. [^{3}H]Catecholamines were eluted from the Amberlite column with 3 ml 4% boric acid. The effluent from the Dowex-50 column plus 2·0 ml H_2O-wash contained the ^3H-H_2O. One ml aliquots of the boric acid eluate, and Dowex-50 effluent were combined with 10 ml Instagel in counting-vials and counted in a Packard Tricarb.

The blank values were normal assay mixtures containing about 1 mM 3-iodo-tyrosine. In the [^{3}H]catecholamine assay such blank values were essentially zero, while in the ^3H-H_2O assay they were consistantly 20 to 25% of the control values. Comparing the two assay-methods it can be seen that about twice as much "^3H-H_2O" as "[^{3}H]dopamine" is formed. The "^3H-H_2O" fraction may also contain the acidic dopamine metabolites [^{3}H]dihydroxyphenylacetic acid (DOPAC) and [^{3}H]homovanillic acid (HVA).

Apomorphine proved to be a potent inhibitor of synaptosomal tyrosine hydroxylase (IC50 about 0·6 μM) with both assay methods described above (see Table 1). The simultaneous addition of haloperidol in concentrations ranging from 0·1 to 0·5 μM increased tyrosine hydroxylase activity to a maximum value lying about half way between that obtained with apomorphine alone and the control. These concentrations of haloperidol alone had little or no effect on tyrosine hydroxylase activity, but concentrations much above 1 μM were inhibitory.

Goldstein & others (1970) carried out similar experiments with striatal slices but were unable to show any reversal of apomorphine inhibition with haloperidol added *in vitro*. However, haloperidol administered *in vivo* to rats shortly before killing resulted in a stimulation of tyrosine hydroxylase activity in striatal slices (Goldstein,

Anagnoste & Shirron, 1973). Cheramy & others (1970) also found an acceleration of tyrosine hydroxylase activity in striatal slices by another neuroleptic, thioproperazine when administered *in vivo* but not when added *in vitro*. The difference between our results and those of Goldstein & others (1970) may be due to the relatively slow diffusion of haloperidol into striatal slices compared with a much more rapid action on the more dispersed synaptosome system.

Similarly, the stimulation of tyrosine hydroxylase activity in striatal slices after *in vivo* administration of thioproperazine (Cheramy & others, 1970) or haloperidol (Goldstein & others, 1973) may be due to blockade of the inhibitory action of dopamine which might accumulate near the dopamine terminals because of slow diffusion out of the slices. Presumably, dopamine leaking out of synaptosomes will diffuse much more rapidly into the surrounding medium.

The failure of haloperidol to reverse completely apomorphine inhibition might be due to a double action of apomorphine on tyrosine hydroxylase, only one of the actions being blocked by haloperidol. For example apomorphine may inhibit synaptosomal tyrosine hydroxylase both directly and indirectly by activating the postulated presynaptic inhibitory dopamine receptor.

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REFERENCES

ANDÉN, N.-E., CORRODI, H. & FUXE, K. (1972). *J. Pharm. Pharmac.*, **24**, 177–182.
ANDÉN, N.-E., MAGNUSSON, T. & STOCK, G. (1973). *Arch. Pharmac.*, **278**, 363–372.
ANDÉN, N.-E., ROOS, B.-E. & WERDINIUS, B. (1964). *Life Sci.*, **3**, 149–158.
ANDÉN, N.-E., RUBENSON, A., FUXE, K. & HÖKFELT, T. (1967). *J. Pharm. Pharmac.*, **19**, 627–629.
BUNNEY, B. S. & AGHAJANIAN, G. K. (1973). *Life Sci.*, **13**, xiii.
BUNNEY, B. S., AGHAJANIAN, G. K. & ROTH, R. H. (1973). *Nature New Biology*, **245**, 123–125.
BUNNEY, B. S., WALTERS, J. R., ROTH, R. H. & AGHAJANIAN, G. K. (1973). *J. Pharmac. exp. Ther.*, **185**, 560–571.
CARLSSON, A. & LINDQVIST, M. (1963). *Acta pharmac. tox.*, **20**, 140–144.
CHERAMY, A., BESSON, M. J. & GLOWINSKI, J. (1970). *Eur. J. Pharmac.*, **10**, 206–214.
CICERO, T. J., SHARPE, L. G., ROBINS, E. & GROTE, S. S. (1972). *J. Neurochem.*, **19**, 2241–2243.
GOLDSTEIN, M., ANAGNOSTE, B. & SHIRRON, C. (1973). *J. Pharm. Pharmac.*, **25**, 348–351.
GOLDSTEIN, M., FREEDMAN, L. S. & BACKSTROM, T. (1970). *Ibid.*, **22**, 715–717.
KAROBATH, M. (1971). *Proc. Nat. Acad. Sci.*, **68**, 2370–2373.
KEHR, W., CARLSSON, A., LINDQVIST, M., MAGNUSSON, T. & ATACK, C. (1972). *J. Pharm. Pharmac.*, **24**, 744–747.
KUHAR, M. J., ROTH, R. H. & AGHAJANIAN, G. K. (1972). *J. Pharmac. exp. Ther.*, **181**, 36–45.
LAHTI, R. A., MCALLISTER, B. & WOZNIAK, J. (1972). *Life Sci.*, **11**, Part I, 605–613.
MCGEER, P. L., BAGCHI, S. P. & MCGEER, E. G. (1965). *Ibid.*, **4**, 1859–1867.
NAGATSU, T., LEVITT, M. & UDENFRIEND, S. (1964). *Analyt. Biochem.*, **9**, 122–126.
NYBÄCK, H., SCHUBERT, J. & SEDVALL, G. (1970). *J. Pharm. Pharmac.*, **22**, 622–624.
RENZINI, V., BRUNORI, C. A. & VALORI, C. (1970). *Clin. Chim. Acta*, **30**, 587–594.
ROOS, B.-E. (1969). *J. Pharm. Pharmac.*, **21**, 263–264.
WALTERS, J. R., ROTH, R. H. & AGHAJANIAN, G. K. (1973). *J. Pharmac. exp. Ther.*, **186**, 630–639.