Neuroleptic interaction with the serotoninergic-dopaminergic mechanisms in the nucleus accumbens

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A locomotor hyperactivity can be induced by dopamine from the nucleus accumbens (Pijnenburg & van Rossum, 1973; Costall & Naylor, 1975; Jackson, Andén & Dahlström, 1975). This response is specifically inhibited by the neuroleptic agents and it has been considered that such an effect may reflect the dopamine antagonistic properties of the neuroleptics (Pijnenburg, Honig & van Rossum, 1975; Costall, Naylor & Pinder, 1976). However, although this hypothesis may appear reasonable for the typical neuroleptics, such as fluphenazine or haloperidol, it may be questioned by the action of the atypical neuroleptics, clozapine, sulpiride and thioridazine. These agents are also effective antagonists of the dopamine hyperactivity induced from the nucleus accumbens (Costall & Naylor, 1976), but whilst they may exert some direct effect on the dopamine receptor, this is generally considered to be rather weak (Leysen, Gommeren & Laduron, 1978).

A consideration of neuroleptic action in the nucleus accumbens purely in terms of dopamine may be misleading, particularly since it has been shown that motor control from this area involves a critical balance between dopamine and 5-HT. This has been indicated biochemically where intra-accumbens 5-HT has been shown to cause a compensatory increase in dopamine release and vice-versa (Pycock, Horton & Carter, 1978) and behaviourally, where the powerful facilitatory effect of dopamine on motor activity is antagonized by 5-HT (Costall, Marsden & others, 1976; Pycock & others, 1978). These observations gain significance when one considers that a weak dopamine antagonist such as clozapine can enhance 5-HT turnover (Burki, Ruch & Asper, 1975; Ruch, Asper & Burki, 1976; Loew, Depoortere & Burki, 1976). Therefore, the present study uses 5-HT agonist and antagonists to analyse the possible contribution of 5-HT mechanisms to the antagonistic effects of neuroleptics against the dopamine hyperactivity from the accumbens.

Bilateral stainless steel guide cannulae (0·65 mm diameter) were chronically implanted, using stereotaxic techniques, to allow the direct injection into the nucleus accumbens of male, Sprague-Dawley rats weighing 300 ± 25 g. Chloral hydrate (300 mg kg^{-1}, i.p.) was used as anaesthetic. 0·3 mm diameter stainless steel stylets terminated 0·5 mm below the guide tips and kept the guides patent. Animals were subjected to intracerebral injections 10–14 days after surgery when they were manually restrained as the stylets were replaced by stainless steel injection units which terminated 2·5 mm below the guides at the 'centre' of the nucleus accumbens (Ant. 9·0, Vert. 0·0, Lat. ± 1·6) (De Groot, 1959). Injection units were coupled to Agla micrometer syringes and 1 μl drug or solvent solution was delivered bilaterally over 5 s with a further 55 s allowed for deposition of drug. Rats were used on two occasions only, with an intervening 3 week recovery period, and were then killed for histological examination. All cannulae locations were found to be correct for injection into the area of the nucleus accumbens.

Activity experiments were carried out between 08·00–18.00 h in a sound-proofed, diffusely illuminated room at 21 ± 3°. Activity boxes (30 cm × 20 cm × 15 cm high) were fitted with photocells. Activity was measured by counting the number of interruptions of the light beam occurring during each 5 min period. Animals were placed in the boxes immediately following the injection of drug or solvent into the nucleus accumbens and activity counts recorded for a maximum of 7 h. All animals were given nialamide (100 mg kg^{-1}, i.p. 2 h) before the intracerebral injection.

Dopamine hydrochloride (Koch-Light) was prepared for intracerebral injection in nitrogen bubbled distilled water neutralized with sodium bicarbonate, 5-hydroxytryptamine bimaleinate (Koch-Light) was dissolved in distilled water: both were prepared immediately before use. For intraperitoneal injection, methysergide hydrogen maleinate (Sandoz) and thioridazine hydrochloride (Sandoz) were prepared in distilled water, haloperidol (Janssen) in 1% lactic acid, sulpiride (Delagrange) and clozapine (Sandoz) in a minimum quantity of hydrochloric acid made up to volume with distilled water, and cyproheptadine hydrochloride (Merck, Sharp and Dohme) in a minimum quantity of *NN*-dimethylformamide made up to volume with distilled water. All doses were calculated as the base. A dose-dependent hyperactivity was induced in rats by 3·125–50 μg dopamine injected bilaterally into the nucleus accumbens in the presence of nialamide (100 mg kg^{-1}, i.p. 2 h before) (approximately 12–73 counts/5 min being the maximum effects for doses 3·125–50 μg dopamine respectively). The hyperactivity reached maximum intensity within 60–90 min for all doses, and the maximum intensity was maintained from 2–6+ h at doses of 3·125–50 μg dopamine respectively. The total duration of effect was at least 6 h for all doses. The threshold and maximum effects, induced respectively by 3·125 and 50 μg dopamine, were not modified by the intraperitoneal administration of either cyproheptadine (1·25–5·0 mg kg^{-1}) or methysergide (0·125–1·0 mg kg^{-1}), administered 2·5 h after the dopamine injection, but 5-HT, injected bilaterally into the nucleus accumbens 2·5 h after dopamine, caused a dose-dependent decrease in the hyperactivity

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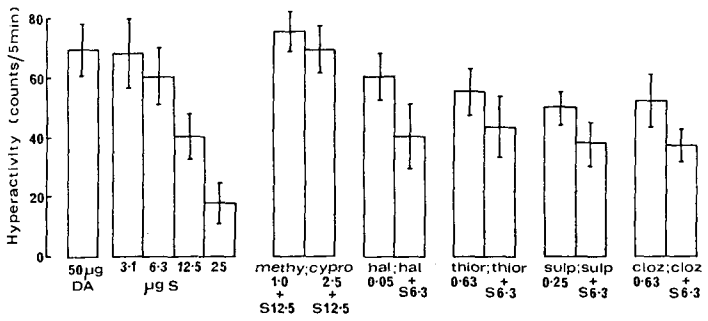

FIG. 1. Dose-dependent antagonism by 5-HT (S) (3·1–25 μg administered bilaterally into the nucleus accumbens) of the hyperactivity induced by intra-accumbens dopamine (DA) (50 μg), reversal of this antagonism by intraperitoneal methysergide (methy) and cyproheptadine (cypro), and the effect of combining a threshold dose of 5-HT (6·3 μg) with threshold doses of the neuroleptic agents haloperidol (hal), thioridazine (thior), sulpiride (sulp) and clozapine (cloz) administered i.p. For the latter experiments control animals were treated with dopamine alone, 5-HT alone, dopamine and 5-HT, dopamine and neuroleptic, and the experimental group with dopamine, 5-HT and neuroleptic. Data for the last two groups of animals is shown. The mean maximum hyperactivity response is presented, in counts/5 min, for dopamine alone or in combination with the treatments indicated. At least 6 animals were used for each drug treatment(s). The mean maximum responses are shown ± s.e.m.

(Fig. 1). This effect of 5-HT was antagonized by a 30 min pretreatment with cyproheptadine (1·25–5·0 mg kg^{-1}) or methysergide (0·125–1·0 mg kg^{-1}).

Neuroleptic agents, administered intraperitoneally 2 h after the bilateral injection of 50 μg dopamine into the nucleus accumbens, antagonized the hyperactivity in a dose-dependent manner. Haloperidol was effective from 0·05–0·4 mg kg^{-1}, hyperactivity being completely inhibited at the larger dose. Thioridazine was similarly effective in a dose range of 0·63–10 mg kg^{-1}, sulpiride at 1·25–20 mg kg^{-1} and clozapine at 0·63–5·0 mg kg^{-1}. Threshold doses of these neuroleptic agents, given intraperitoneally, were combined with a threshold dose of 5-HT (6·3 μg) administered bilaterally into the nucleus accumbens. The effects of such combinations were no greater than could be expected from addition (Fig. 1). In a further series of experiments two doses of each neuroleptic were selected which caused a moderate and marked reduction in the dopamine hyperactivity. These reductions in activity were antagonized by methysergide and cyproheptadine, administered peripherally as a 30 min pretreatment (Fig. 2).

The ability of intra-accumbens 5-HT to antagonize the hyperactivity induced by dopamine from the same area indicates a functionally antagonistic role for forebrain serotoninergic and dopaminergic systems in locomotor control. In doses shown to antagonize the locomotor depressant effects of 5-HT, cyproheptadine and methysergide reduced the ability of typical and atypical neuroleptic agents to antagonize the dopamine hyperactivity (see also Costall, Fortune & Naylor, 1977). If one accepts that, at the doses used, cyproheptadine and methysergide do not significantly affect other neurotransmitter systems, then it could be hypothesized that the inhibitory effects of neuroleptic agents may involve an enhanced 5-HT activity. Since 5-HT and dopamine were administered directly into the nucleus accumbens, it may be proposed that this is their site of

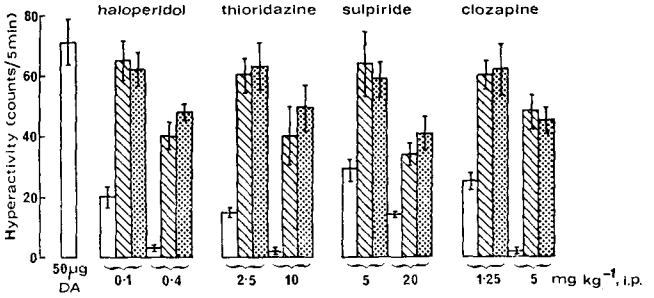

FIG. 2. Effect of 5-HT antagonists on the neuroleptic depression of hyperactivity induced by 50 μg dopamine (DA) administered bilaterally into the nucleus accumbens. Two doses of each neuroleptic (open columns) were administered intraperitoneally in the presence of 1·0 mg kg^{-1}(i.p.) methysergide (hatched columns) or 2·5 mg kg^{-1} (i.p.) cyproheptadine (stippled columns). The neuroleptics were administered at a time of maximal dopamine effect, 30 min after methysergide or cyproheptadine. The mean maximum hyperactivity response is presented, in counts/5 min, for dopamine alone or in combination with the treatments indicated. At least 6 animals were used at each drug treatment(s). The mean maximum response is shown ± s.e.m.

interaction, and this area may also be the site at which the 5-HT antagonists, administered peripherally, are also exerting their effects, although other forebrain areas cannot be ignored. The inability of methysergide and cyproheptadine to completely antagonize the inhibitory action of the larger doses of neuroleptic agents suggests that the reduction in dopamine hyperactivity is not due solely to an enhanced 5-HT activity. Nevertheless, the indication that an enhanced 5-HT function may be important for the action of neuroleptic drugs to inhibit a dopamine response is of interest, particularly for the atypical agents, clozapine, sulpiride and thioridazine. These agents are classified as atypical since they generally fail to cause a marked effect in behavioural tests considered to reflect dopamine blockade, and yet the biochemical experiments to determine the action of these compounds to modify mesolimbic function have invariably assumed a primary drug action on dopamine mechanisms. Thus, the apparent dissociation of extrapyramidal effects (striatal action) from an antipsychotic action (mesolimbic system) using the atypical agents has been investigated experimentally by attempting to correlate a differential action on dopamine turnover in the two areas (Waldmeier & Maitre, 1976; Westerink, Lejeune & van Pragg, 1977). Generally, whilst some differences have been found the degrees of difference are small. It is suggested that future studies should additionally consider an involvement of 5-HT within the two areas.

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REFERENCES

BURKI, H. R., RUCH, W. & ASPER, H. (1975). *Psychopharmacologia (Berl.)*, **41**, 27–33.
COSTALL, B. & NAYLOR, R. J. (1975). *Eur. J. Pharmac.*, **32**, 87–92.
COSTALL, B. & NAYLOR, R. J. (1976). *Ibid.*, **35**, 161–168.
COSTALL, B., FORTUNE, D. H. & NAYLOR, R. J. (1977). *Br. J. Pharmac.*, **60**, 266P.
COSTALL, B., MARSDEN, C. D., NAYLOR, R. J. & PYCOCK, C. J. (1976). *J. Pharm. Pharmac.*, **28**, 523–526.
COSTALL, B., NAYLOR, R. J. & PINDER, R. M. (1976). *Psychopharmacology*, **48**, 225–231.
DE GROOT, J. (1959). *Verh. K. Ned. Akad. Wet.*, **52**, 14–39.
JACKSON, D. M., ANDÉN, N.-E. & DAHLSTRÖM, A. (1975). *Psychopharmacologia (Berl.)*, **45**, 139–149.
LEYSEN, J. E., GOMMEREN, W. & LADURON, P. M. (1978). *Biochem. Pharmac.*, in the press.
LOEW, D. M., DEPOORTERE, H. & BURKI, H. R. (1976). *Arzneimittel-Forsch.*, **26**, 1080–1083.
PIJNENBURG, A. J. J., HONIG, W. M. M. & VAN ROSSUM, J. M. (1975). *Psychopharmacologia (Berl.)*, **41**, 175–180.
PIJNENBURG, A. J. J. & VAN ROSSUM, J. M. (1973). *J. Pharm. Pharmac.*, **25**, 1003–1005.
PYCOCK, C. J., HORTON, R. W. & CARTER, C. J. (1978). *Int. Soc. for Neurochemistry, Satellite Symposium Dopamine* Southampton, U.K., Aug 30th–1st Sept., Editors, Iversen, L., Roberts, P. J. and Woodruff, G. N. New York: Raven Press, in the press.
RUCH, W., ASPER, H. & BURKI, H. R. (1976). *Psychopharmacologia (Berl.)*, **46**, 103–109.
WALDMEIER, P. C. & MAITRE, L. (1976). *J. Neurochem.*, **27**, 589–597.
WESTERINK, B. H. C., LEJEUNE, B. & VAN PRAAG, H. M. (1977). *Eur. J. Pharmac.*, **42**, 179–190.

Receptor interaction for the α-antagonist WB4101 (2-(*N*[2,6-dimethyoxyphenoxyethyl])amino-methyl-1,4-benzodioxane)

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WB4101, a member of the series of benzodioxanes described by Fenton, Green & others (1965) has been evaluated for α-adrenoceptor blocking activity (Mottram & Kapur, 1975). The results indicated that it produced profound post-synaptic α-antagonism against noradrenaline on rat vas deferens, having a pA_2 value (Schild, 1947) of 9·8. Likewise, it has been shown to possess potent α-antagonistic properties in the central nervous system. (Greenberg, U'Prichard & Snyder, 1976). Mottram & Kapur (1975) have suggested that the interaction between WB4101 and the α-receptor involves subsites within the receptor for the nitrogen atom, the benzodioxan moiety and also a possible aromatic subsite though which the dimethoxy benzene may interact. This tertiary interaction may account for the very high potency of WB4101.

To elucidate further the receptor site, a series of benzodioxanes previously described by Green, Shapero & Wilson, 1969 was chosen (1) to study the effect of altering the chain length between amine and aromatic groups, and (2) to examine the effect of ring substitution on drug-receptor interaction.

Male Wistar rats (200–300 g) were killed by a blow to the head, their vasa deferentia removed and stripped

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