Atropine manipulation of elevated cerebral dopamine turnover caused by haloperidol or substituted benzamide drugs

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High concentrations of acetylcholine, choline acetyltransferase and cholinesterase are found in the striatum, probably within a system of cholinergic interneurons (McGeer & others, 1975; Butcher, 1977). These cholinergic interneurons appear to be inhibited, functionally, by the dopaminergic nigrostriatal pathway. Thus, neuroleptic drugs, which are dopamine antagonists, have been shown to decrease striatal acetylcholine concentrations and increase acetylcholine release (Stadler, Lloyd & others, 1973; Consolo, Ladinsky & Garattini, 1974; Consolo, Ladinsky & Bianchi, 1975; Consolo, Ladinsky & others, 1977). The reverse is also true in that the dopamine nigrostriatal pathway appears to be inhibited functionally by a cholinergic input. Thus, neuroleptic-induced increases in striatal dopamine turnover can be reversed, at least partially, by the administration of antiacetylcholine drugs (O'Keefe, Sharman & Vogt, 1970; Andén, 1972; Corrodi, Fuxe & Lidbrink, 1972; Bartholini, Keller & Pletscher, 1975).

In another dopamine containing area, the mesolimbic system, high concentrations of acetylcholine also are present. However, in contrast to the striatum, the relation between dopaminergic and cholinergic pathways within the mesolimbic area is unclear. Thus, while administration of oxotremorine or physostigmine results in an increase in limbic (and striatal) homovanillic acid (HVA) concentrations, effects reversible by trihexiphenidyl (Andén, 1974), dopamine agonists and antagonists failed to alter acetylcholine concentrations or release in the tuberculum olfactorium and nucleus accumbens in doses effective in the striatum (Stadler, Gadea-Ciria & Bartholini, 1975; Consolo & others, 1977). Further, neuroleptic-induced increases in mesolimbic HVA concentrations have been reported not to be reversed by antiacetylcholine drugs (Andén, 1972).

Substituted benzamide drugs such as sulpiride and metoclopramide represent an unusual group of dopamine receptor antagonists. Thus, while blocking locomotor activity induced by apomorphine, inhibiting apomorphine- and amphetamine-induced circling behaviour and increasing both striatal and mesolimbic dopamine turnover, these compounds have little cataleptic activity, do not inhibit dopamine stimulated adenylate cyclase in rat striatal preparations, and only weakly inhibit [^{3}H]haloperidol receptor binding in this area (Dolphin, Jenner & others, 1975; Peringer, Jenner & Marsden, 1975; Peringer, Jenner & others, 1976; Elliott, Jenner & others, 1977; Jenner, Elliott & others, 1978). Since these compounds appear to intereact with cerebral dopamine pathways in a manner which differs from that of classical neuroleptics it was of interest to determine if the actions of such drugs also are modified by cholinergic manipulation. A suggestion that substituted benzamide drugs might behave differently was provided by the previous finding that atropine did not reverse the increase in striatal HVA produced by metoclopramide (Ahtee, 1975). We now report the effect of atropine on the increase in dopamine turnover produced by six substituted benzamide drugs, administered in doses known to produce behavioural effects and alter whole brain dopamine turnover, as judged by raised concentrations of both striatal and mesolimbic homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC).

Male Swiss S or P strain mice (20–25 g; Animal Suppliers Ltd.) were injected intraperitoneally with either saline (0·1 ml 0·9% sodium chloride), haloperidol (0·1 mg kg^{-1}; Serenace; Searle Ltd.), metoclopramide (20 mg kg^{-1}; *N*-[diethylaminoethyl]-2-methoxy-4-amino-5-chlorobenzamide hydrochloride), sulpiride (50 mg kg^{-1}; *N*-[1′-ethyl-2′-pyrrolidinylmethyl] -2-methoxy sulphamoyl benzamide), tiapride (50 mg kg^{-1}; *N*-[diethylaminoethyl]-2-methoxy-5-methylsulphonyl benzamide), sultopride (10 mg kg^{-1}; *N*-[1-ethyl-2-pyrrolidinylmethyl]-2-methoxy-5-ethylsulphonyl benzamide) (all supplied by SESIF France), clebopride as the malic acid salt (10 mg kg^{-1}; *N*-[N′-benzylpiperidin-4′-yl]-4-amino-5-chloro-2-methoxy benzamide; Almirall Laboratories Ltd.) or tigan (200 mg kg^{-1}; *N*-[(2′-dimethylaminoethoxy) benzyl]-3,4,5-trimethoxy benzamide; Roche Products Ltd.). At the same time some animals received a subsequent intraperitoneal injection of saline (0·1 ml) or atropine sulphate (50 mg kg^{-1}; Sigma Chemical Co.). The doses of benzamide drugs used had previously been shown to alter dopamine turnover and to affect dopamine-induced behaviour.

Mice were killed by cervical dislocation 2 h following drugs administration and the brain rapidly removed and cooled to −20°. The striata and a mesolimbic slice were removed as previously described (Peringer & others, 1975). The mesolimbic slice contained the corpora amygdala, the olfactory tubercle and the nucleus accumbens. Parts from two mice were pooled and striatal and mesolimbic HVA and DOPAC con-

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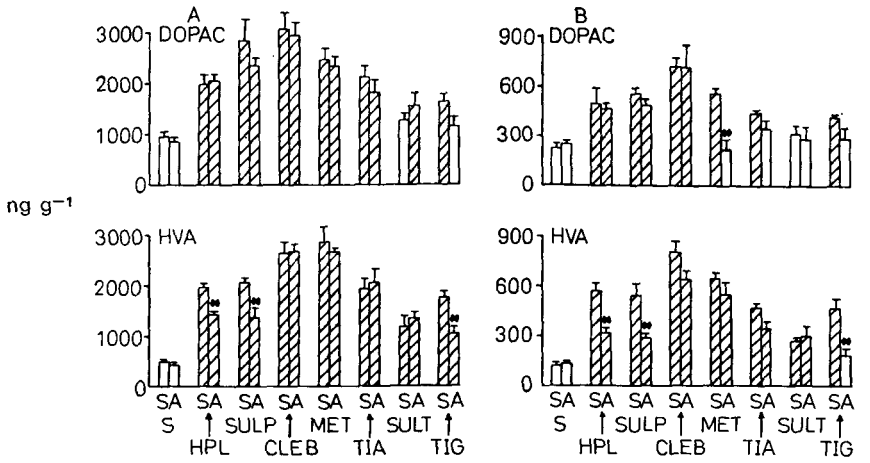

FIG. 1. The influence of atropine (50 mg kg^{-1}) on the elevation of A, striatal DOPAC and HVA and B, mesolimbic DOPAC and HVA produced by the administration of either haloperidol or some substituted benzamide drugs to mice. Mice were injected intraperitoneally with saline (S; 0·1 ml), haloperidol (HPL; 0·1 mg kg^{-1}), metopramide (MET; 20 mg kg^{-1}), sulpiride (SULP; 50 mg kg^{-1}), tiapride (TIA; 50 mg kg^{-1}), sultopride (SULT; 10 mg kg^{-1}), clebopride (CLEB; 10 mg kg^{-1}) or tigan (TIG: 200 mg kg^{-1}) 2 h before death. Atropine (A; 50 mg kg^{-1}) was administered at the same time to some groups of animals. Each value is the mean (±s.e.) of at least 4 determinations. The results were analysed by Student's *t*-test. Open columns show values not different ($P>0·05$) from saline controls, hatched columns indicate values differing ($P<0·05$) from the appropriate saline control group and an asterisk indicates the ability of atropine to reverse ($P<0·05$) the drug-induced elevations of HVA or DOPAC compared with animals receiving haloperidol or a benzamide drug alone.

centrations were determined by the technique of Murphy, Robinson & Sharman (1969).

The administration of atropine (50 mg kg^{-1}) 2 h before death produced no changes in the concentrations of striatal and mesolimbic HVA or DOPAC (Fig. 1). Haloperidol (0·1 mg kg^{-1}), as expected, produced a marked rise in the concentration of the dopamine metabolites in both striatal and mesolimbic areas in agreement with previous findings (Andén, Roos & Werdinius, 1964). Concomitant administration of atropine partially reversed the increase in striatal and mesolimbic HVA concentrations but was without effect on the elevated concentrations of DOPAC. While our findings in the striatal area are in agreement with previous reports (Andén, 1972), the atropine-induced reversal of the neuroleptic-induced increase in HVA in the mesolimbic area contrasts with the failure of trihexiphenidyl to produce a similar reversal in the rat and rabbit limbic system (Andén, 1972; Bartholini & others, 1975) and with the reported lack of a dopaminergic-cholinergic link in the rat nucleus accumbens septi and tuberculum olfactorium (Consolo & others, 1977).

These data confirm the view that antiacetylcholine drugs can at least partially reverse the increase in cerebral HVA concentrations caused by neuroleptic compounds. Why atropine does not produce similar changes in striatal and mesolimbic DOPAC concentrations is unclear. HVA has been associated with extraneuronal dopamine metabolism while DOPAC is thought to represent intraneuronal breakdown (Fry & Sharman, 1976). It could be considered therefore that the action of atropine is more concerned with utilization of dopamine (as judged by decreased HVA concentrations) than with reducing dopamine synthesis (as judged by unchanged DOPAC concentrations). This view is confirmed by the lack of effect of atropine on dopa accumulation following pretreatment of animals with a central decarboxylase inhibitor, indicating no change in dopamine synthesis (Javoy, Agid & Glowinski, 1975). However, the same authors showed that atropine increased accumulation of [^{3}H]dopamine from [^{3}H]tyrosine without altering steady state dopamine concentrations, suggesting the drug reduced the utilization of the newly synthezised amine. Such an action might also explain the ability of antiacetylcholine drugs to potentiate amphetamine-induced, but not apomorphine-induced, circling behaviour by postulating the presence of an increased pool of newly synthesized dopamine available for release (Pycock, Milson & others, 1978).

Sulpiride (50 mg kg^{-1}), clebopride (10 mg kg^{-1}), metoclopramide (20 mg kg^{-1}), tiapride (50 mg kg^{-1}), and tigan (200 mg kg^{-1}) all produced an increase in striatal and mesolimbic HVA and DOPAC concentrations (Fig. 1). Sultopride (10 mg kg^{-1}), however, while increasing HVA concentrations failed to elevate striatal and mesolimbic DOPAC concentrations. The increases observed, taken with previous studies of functional dopamine antagonism are assumed to represent the ability of these compounds in these doses to block cerebral dopamine receptors.

The co-administration of atropine with the antipsychotic sulpiride produced a partial reversal of the increase in striatal and mesolimbic HVA concentrations as seen with haloperidol. Again no change was seen in cerebral DOPAC concentrations. In contrast, administration of atropine with the more potent benzamide clebopride, a compound of as yet unknown clinical use, failed to cause a reversal of the increase in HVA or DOPAC in either dopamine containing area. Further, following metoclopramide administration atropine produced no reversal of the HVA increases, although a decrease in mesolimbic DOPAC concentrations was observed. Similarly, while atropine failed to reverse the increased HVA concentrations caused by tiapride, tiapride no longer produced an increase in mesolimbic DOPAC concentrations when compared with animals receiving atropine alone. These latter drugs have no known antipsychotic activity, indeed, tiapride is thought useful in treating dyskinetic phenomena.

The combination of atropine with sultopride again failed to reverse the sultopride-induced increase in striatal and mesolimbic HVA concentrations. However, while sultopride alone did not elevate either striatal or mesolimbic DOPAC concentrations, in the presence of atropine sultopride produced an increase in striatal DOPAC compared with animals receiving atropine alone. Sultopride has antipsychotic activity but in contrast to other benzamides produces an incidence of extrapyramidal side-effects similar to classical antipsychotic compounds.

Tigan differed from the other compounds examined in that following concomitant administration of atropine, a total abolition of the increased mesolimbic and striatal DOPAC and mesolimbic HVA concentrations was observed together with a marked reduction in striatal HVA. Tigan differs in structure from the other benzamides tested and is relatively weak in elevating HVA and DOPAC concentrations and in inhibiting dopamine mediated behaviours (Elliott & others, 1977). Indeed, it has been shown that tigan, unlike other benzamides, is a potent inhibitor of acetylcholinesterase activity (Huizing, 1977). This evidence would therefore suggest that tigan, rather than acting on cerebral dopamine receptors directly, may act indirectly by increasing cholinergic activity, an action which can be almost totally reversed by anti-acetylcholine drugs such as atropine. Indeed, sufficient doubt surrounds the mode of action of tigan, in relation to other benzamide drugs, for us not to consider it further in the discussion of the present work.

It would appear from the present study that most substituted benzamide drugs differ from classical neuroleptics such as haloperidol in the manner in which they interact with cerebral dopamine pathways in that the major part of their activity is exerted on a part of the dopamine pathways that is not balanced by a cholinergic input. If, as seems likely, substituted benzamide drugs act on a post-synaptic dopamine receptor it can be postulated that the receptor is different from that modulated by a cholinergic input. Thus, while the presence of an interaction between cholinergic and dopaminergic neurons is established, there is no evidence to suggest this is necessarily true for all dopamine systems and their receptors. Indeed, early fluorescent histochemical studies revealed the presence of different types of dopamine fibre tracts, the functional significance of which remains to be determined (Olson, Seiger & Fuxe, 1972). In this respect it is particularly interesting to note that even the increase in HVA concentrations caused by haloperidol are only partially reversed by acetylcholine drugs. This might suggest, therefore, that the difference between classical neuroleptics such as haloperidol and the substituted benzamide drugs lies in the ability of the former to interact with all parts of the cerebral dopamine pathways while the latter are restricted in action to one or more parts of the system. The inability of metoclopramide (or sulpiride) to further elevate the maximal change in striatal HVA induced by haloperidol (Westerink, Lejeune & others, 1977) suggests that haloperidol itself fully activates those dopamine mechanisms engaged by substituted benzamides. The anatomical basis for the presence of atropine sensitive and non-sensitive increases in dopamine metabolite concentrations, apparently present in both striatal and mesolimbic areas, remains to be established.

Sulpiride provides a major exception to the above discussion since the increased concentrations of dopamine metabolites produced by this drug showed atropine sensitivity identical to haloperidol. Sulpiride is the only clinically useful antipsychotic benzamide identified so far and it would be tempting to speculate on the relevance of the changes observed to antipsychotic activity.

The difference in biochemical interaction with anti-acetylcholine drugs between sulpiride and the other benzamides tested demonstrates differences between members of this series. Such differences have also been highlighted in functional terms. Thus, while a threshold cataleptic dose of sulpiride synergizes with threshold doses of the acetylcholine-like drug RS86 (spiro- *N'*-methyl-piperidyl-4'- ethylsuccinimide), this does not occur with metoclopramide (Costall & Naylor, 1973). Also, while sulpiride inhibits hyperactivity caused by injection of dopamine into the nucleus accumbens, metoclopramide does not (Costall & Naylor, 1976). Whether the non-atropine sensitive increase in the concentration of dopamine metabolites caused by benzamide drugs is of such behavioural or even clinical significance is not known. However, while atropine was unable to reverse the striatal HVA increase due to metoclopramide, it did antagonize metoclopramide-induced catalepsy (Ahtee, 1975).

In conclusion the present study demonstrates a difference in the degree to which the increase in dop-

amine turnover caused by haloperidol and many substituted benzamide drugs is sensitive to antiacetylcholine drugs. This observation is further evidence that some of these compounds act differently on cerebral dopamine pathways compared with classical antipsychotics.

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