The use of scopolamine for the estimation of the central antiacetylcholine properties of neuroleptics*

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The antagonistic effect of antiacetylcholine (antiACh) substances on the increase in dopamine (DA) turnover caused by neuroleptic drugs has been reported by various authors (O'Keeffe et al 1970; Perez-Cruet et al 1971; Andén 1972; Bowers & Roth 1972; Bürki et al 1976). Some found a lesser degree of antagonism in the mesolimbic area than in the striatum (Andén 1972; Bartholini et al 1975) while according to Westerink and Korf (1975), this type of interaction was only demonstrable in the mesolimbic area, and not in the striatum. The fact that only single doses of the neuroleptics were used in some of these investigations might explain the apparent discrepancies. Recently, Robinson et al (1977) asserted that the reversal of the effect of neuroleptics on DA turnover by atropine-like drugs is not related to their antiACh properties.

In an effort to resolve these apparent contradictions, we have attempted to quantify the antagonistic effect of a maximally active dose of scopolamine on the increase in tyrosine hydroxylation in vivo elicited by a series of neuroleptic agents of different intrinsic antimuscarinic potency. GP 50 302, an experimental compound of CIBA-GEIGY's (Delini-Stula et al 1978) was included in this study.

Tyrosine hydroxylation was determined by reference to the accumulation of Dopa after central decarboxylase inhibition, as described by Carlsson & Lindqvist (1973). Female Tif: Raif (SPF) rats (Tierfarm Sisseln, Switzerland), 180–220 g, received graded doses of haloperidol (Cilag AG, Schaffhausen, Switzerland), clozapine (Wander AG, Berne, Switzerland), thioridazine (Sandoz AG, Basle, Switzerland), sulpiride (Laboratoires Delagrange, Paris, France), chlorpromazine HCl (synthesized by Dr V. Mychajlyszyn in our Chemistry Department) or GP 50 302 (2-methyl-2, 3-dihydro-1H-dibenzo [2,3:6,7] thiepino [4,5-c] pyrrole methanesulphonate). With the exception of sulpiride, which was administered intraperitoneally, the drugs were given by mouth 1¼ h before the decarboxylase inhibitor, Benzerazide (Ro-4-4602) (800 mg kg^{-1} i.p.), 45 min after which the animals were decapitated. In a parallel series of experiments, animals were treated according to the same schedule with additional injections of scopolamine HBr (Merck, Darmstadt, W. Germany; 10 mg kg^{-1} i.p.), once 3¼ h and once 1 h before the decarboxylase inhibitor, to assure complete blockade of the central muscarinic receptors throughout the experiment. Within 3 min of decapitation, the corpora striata and mesolimbic areas were dissected (for dissection see Waldmeier & Maître 1976) and put into ice-cold 0·4 M perchloric acid. Four striata and 2 mesolimbic areas, respectively, were pooled per sample, and 5 samples per group were used. Dopa was isolated on Dowex 50 W × 4 columns according to Atack & Magnusson (1970) and determined by semi-automated fluorometry (Waldmeier et al 1974). The means of the Dopa values after benzerazide alone varied between 682 and 869 ng g^{-1} in the striatum and between 469 and 933 ng g^{-1} in the mesolimbic area. The standard errors of the mean of these control groups varied between ± 5 and ± 12%.

All the drugs used caused a dose-related increase in tyrosine hydroxylation in both the striatum and the mesolimbic area. The effect of haloperidol and, to a lesser degree, that of clozapine and chlorpromazine, was clearly more pronounced in the striatum than in the mesolimbic area. This difference was less obvious in the tissues from animals treated with thioridazine, sulpiride

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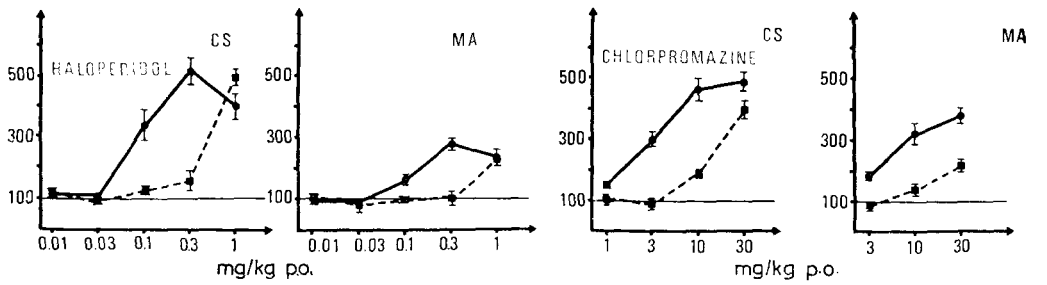

FIG. 1. Effect of scopolamine on the increase in dopa accumulation after central decarboxylase inhibition induced by haloperidol and chlorpromazine in striatum (CS) and mesolimbic area (MA) of the rat. Graded doses of the neuroleptics were given 1¼ h before the decarboxylase inhibitor, Ro-4-4602 (800 mg kg^{-1} i.p.). The animals were decapitated 45 min thereafter (black circles and lines). Other groups of rats were additionally treated twice with scopolamine (10 mg kg^{-1} i.p., 3¼ and 1 h before Ro-4-4602; black squares and broken lines). The points and bars represent means ± s.e.m. (n = 5) in percent of controls.

and GP 50 302. Thus, none of the compounds had a greater effect in the mesolimbic area than in the striatum, confirming our observations in previous experiments performed according to a different method (Waldmeier & Maître 1976).

All the dose-response curves obtained for both the striatum and the mesolimbic area were shifted to the right by scopolamine, but to varying extents (Figs 1–3). The curves of haloperidol and chlorpromazine showed the largest shifts in both areas (Fig. 1). In the striatum the smallest shift was observed with clozapine (Fig. 2) and in the mesolimbic area with sulpiride (Fig. 3). An attempt to summarize these results is presented in Table 1, which shows the ratios of the ED200 values (the dose which doubled dopa accumulation with respect to controls) of each drug with and without the additional administration of scopolamine. A ratio of 1 would mean that the dose-response curve of the neuroleptic is not displaced by the anti-ACh drug and would therefore indicate a strong intrinsic antiacetylcholine effect: a ratio approaching zero suggests weak intrinsic antiACh properties.

As can be seen from Table 1, in the striatum, clozapine has the most potent antiACh effect, followed by GP 50 302 ≥ sulpiride = thioridazine > chlorpromazine = haloperidol. In the mesolimbic area, the order is somewhat different: sulpiride > GP 50 302 > clozapine ≥ thioridazine > haloperidol ≥ chlorpromazine.

The above results demonstrate that the increase in DA turnover produced by neuroleptics with little or no intrinsic antiACh effect is readily antagonized by high doses of scopolamine, both in the mesolimbic area and in the striatum. By contrast, the effects of the strongly antiACh neuroleptics clozapine and thioridazine (Snyder et al 1974; Pearl et al 1976; Sayers & Bürki 1976) are only weakly antagonized by scopolamine. In fact, it seems that the intrinsic antiACh activities of neuroleptic drugs can be ranked according to the factor by which scopolamine shifts their dose-response curves for DA turnover to the right. The order of antiACh potency of the four compounds clozapine, thioridazine, chlorpromazine and haloperidol in our model corresponds fairly well to that observed in other test systems, such as mydriasis, smooth-muscle contraction (Pearl et al 1976; Sayers & Bürki 1976), oxotremorine-induced tremor (Sayers & Bürki 1976) and affinity to muscarinic sites (Snyder et al 1974; Sayers & Bürki 1976).

Table 1. Relative intrinsic antiACh effects in striatum and mesolimbic area.

Drug	ED200(NL) / ED200(NL+sco) striatum	ED200(NL) / ED200(NL+sco) mesolimbic area
Haloperidol	0·14	0·20
Chlorpromazine	0·15	0·16
Thioridazine	0·45	0·31
Sulpiride	0·45	0·71
GP 50 302	0·50	0·50
Clozapine	0·67	0·37

The ED200 values are taken from the dose-response curves shown in Figs 1–3. ED200(NL) is the value obtained with the neuroleptic alone, ED200(NL + sco) that with concomitant scopolamine treatment. A value of one indicates that the effect of the neuroleptic is not changed by scopolamine and suggests marked intrinsic antiACh-like activity.

The ED200 values for GP 50 302 in both areas and for thioridazine + scopolamine in the mesolimbic area were determined by graphical interpolation.

The effects of sulpiride and GP 50 302 which are both virtually devoid of direct antiACh activity (Fjalland et al 1977; Bittiger, personal communication), were only slightly antagonized by scopolamine, which would make it appear that they were endowed with intrinsic antiACh properties. In interpreting these results, it must be borne in mind that GABA-like, baclofen-like and anti-hydroxytryptamine (5-HT) compounds can likewise reduce the effects of neuroleptics on DA turnover (Lahti & Losey 1974; Fuxe et al 1975; Waldmeier et al 1979).

Generally it may be assumed that drugs interfering with any transmitter system in direct communication with dopaminergic cells or situated within the postulated feed-back loop will interfere with the effect of neuroleptic drugs on DA-turnover. Thus it is conceivable that sulpiride and GP 50 302 exert their

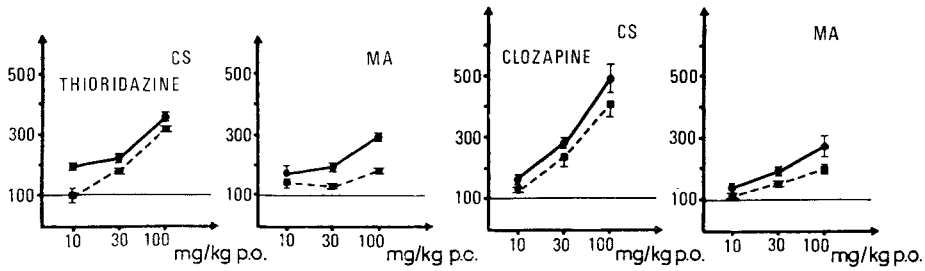

FIG. 2. Effect of scopolamine on the increase in dopa accumulation induced by thioridazine and clozapine in striatum and mesolimbic area (CS and MA, resp.). For explanation, see legend of Fig. 1.

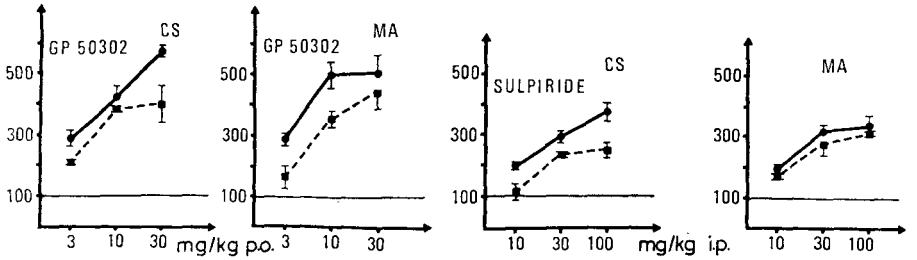

FIG. 3. Effect of scopolamine on the increase in dopa accumulation induced by GP 50302 and sulpiride in striatum and mesolimbic area (CS and MA, resp.). For explanation, see legend of Fig. 1.

apparently antiACh effect in our model via interference with a transmitter system other than the cholinergic system.

In this context, the recent work published by Robinson et al (1977) deserves comment. These authors have compared the dose-response curves for the increase in HVA of the racemate and the (+)- and (—)-enantiomers of cyanocyproheptadine. The (+)-enantiomer displays antiACh but no antidopamine activity, whereas the converse is true of the (—)-enantiomer. They found no difference between the racemate and the (—)-isomer. They concluded that "other factors (than cholinolytic properties) may be responsible for the observed reduction in the concentration of HVA following the combined administration of certain cholinolytic compounds with a neuroleptic drug." However, in their experiments the ratio of antiACh to antidopamine components was the same at all the doses used. Thus, if the antiACh component was too weak relative to the antidopamine component to antagonize the effects of the latter on DA turnover, this would apply throughout the course of the dose-response curve. Certain other findings may also be relevant in this context: methoxycyproheptadine, a structural analogue of cyanocyproheptadine, was found to possess anti-5-HT properties in the (−)-isomeric form and antiACh properties in the (+)-isomeric form (Remy et al 1977). In our view, the possibility should be considered that the antidopaminergic isomer of cyanocyproheptadine also shows anti-5-HT properties. As anti-5-HT agents also antagonize the effects of neuroleptics on DA turnover (Waldmeier et al 1979) it is quite possible that intrinsic anti-5-HT effects of cyanocyproheptadine antagonized the increase in HVA to such a degree that it could not be further antagonized by the antiACh isomer.

In conclusion, the antiACh component of a neuroleptic drug is likely to reduce the effect on DA turnover due to DA receptor blockade. This, however, is not the only component able to produce this effect. In addition, an antiACh drug will attenuate the increase in DA turnover brought about by a DA antagonist, unless the latter possesses intrinsic properties such as antiACh, anti-5-HT, GABA-like or baclofen-like activity, producing a similar effect. January 10, 1979

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