Br. J. Pharmac. (1983), **80**, 581–585

Effects of various antipsychotic drugs upon the striatal concentrations of *para*-hydroxyphenylacetic acid and *meta*-hydroxyphenylacetic acid in the mouse

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1 The endogenous concentrations of *p*- and *m*-hydroxyphenylacetic acid in the mouse caudate nucleus were determined by a gas chromatographic or a gas chromatographic-mass spectrometric technique and the concentrations were about 30 and 11 ng g^{-1} respectively.

2 The subcutaneous administration of (+)-butaclamol (1 mg kg^{-1}), haloperidol (5 mg kg^{-1}), molindone (100 mg kg^{-1}), sulpiride (50 mg kg^{-1}) or chlorpromazine (20 mg kg^{-1}) increased the concentration of mouse striatal *p*- and *m*-hydroxyphenylacetic acid; the effects were observed at 2 h after drug administration.

3 Lower doses of chlorpromazine (2 mg kg^{-1}), haloperidol (0.2 mg kg^{-1}) and molindone (2 mg kg^{-1}) did not affect *p*- or *m*-hydroxyphenylacetic acid concentrations.

4 The time course for the concentration changes produced by chlorpromazine (20 mg kg^{-1}) revealed that the formation of the metabolites occurred within 30 min after its administration and that their efflux from the caudate nucleus took at least 4 h for *p*-hydroxyphenylacetic acid and more than 8 h for *m*-hydroxyphenylacetic acid.

5 Promethazine and (−)-butaclamol which have chemical structures related to chlorpromazine or (+)-butaclamol respectively but which lack antipsychotic activity, produced no effect on striatal *p*- or *m*-hydroxyphenylacetic acid concentrations.

6 The results suggest that antipsychotic drugs increase the utilization of mouse striatal *p*- and *m*-tyramine and that after use the amines are metabolized by monoamine oxidase to form *p*- or *m*-hydroxyphenylacetic acid. The synthesis of the acid metabolites occurs within 30 min after chlorpromazine administration and their efflux from the caudate nucleus takes from 4–8 h.

Introduction

In 1963, Carlsson & Lindquist described an increase in mouse brain 3-methoxytyramine concentrations after the administration of haloperidol or chlorpromazine to animals pretreated with a monoamine oxidase inhibitor. These results were attributed to the receptor blockade caused by the antipsychotic drugs thus leading to increased utilization of the catecholamines and subsequently to increased concentrations of their methoxylated metabolites. Since these authors observed no decrease in the monoamine tissue concentrations, they proposed that these drugs produce neuronal activation and thus stimulate the synthesis of the monoamines. In agreement with these findings it was reported that the concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were increased in the rabbit striatum after administering haloperidol or chlorpromazine (Andén, Roos & Werdinius, 1964).

The administration of clinically efficacious antipsychotic drugs produced a marked decrease in mouse striatal *p*-tyramine concentrations and no change or an increase in *m*-tyramine (Juorio, 1977; 1979; 1980a). The existence of an inverse relationship between *p*- or *m*-tyramine concentrations and dopamine metabolism was proposed (Juorio, 1980b).

This is an investigation of the effect of some antipsychotic drugs on the concentration of *p* or *m*-

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hydroxyphenylacetic acid (*p*- or *m*-HPAA) in the mouse caudate nucleus. For comparison, the effects of some structurally related non-antipsychotic compounds were also investigated.

Methods

Male Swiss mice (body weight 20–25 g) were stunned, decapitated and their brains rapidly removed. The caudate nuclei were dissected and rapidly frozen on dry ice.

The tissue was homogenized in zinc sulphate containing 250 ng of *para*-hydroxyphenylpropionic acid, the g.c. internal standard, or 50 ng each of the deuterated acids (g.c.-m.s. internal standards). Equal volumes of barium hydroxide were added to precipitate protein, sulphate and endogenous phosphates. The supernatant obtained after centrifugation was percolated through a DEAE-Sephadex column and the acid fraction eluted from the column using 1.5 M pyridinium acetate buffer. The acids were extracted from the buffer with ethyl acetate, derivatized using pentafluoropropionic anhydride (0.05 ml) and hexafluoroisopropanol (0.15 ml) and heated for 90 min at 55°C. Excess derivatizing reagent was removed by washing the hexane solution (added after heating) with large volumes of 1.0 M phosphate buffer (pH 6.0). One μl of the hexane solution was injected into a gas chromatograph (Hewlett-Packard 5840A) equipped with an electron capture detector and a capillary inlet system. The column employed was a Scientific Glass Engineering Ltd. (Melbourne, Australia) D class, support coated open tubular (SCOT) column, 50 m long and coated with SP-2100 (SE 30). Further details of this procedure have been published (McQuade, Juorio & Boulton, 1981).

The acids were also quantified by using a g.c.-m.s. technique. The major differences between this assay method and the g.c. procedure were the use of a deuterated internal standard, the deletion of the DEAE-Sephadex isolation and the use of a different derivative. The acids were methylated using methanolic HCl (Karoum, Gillin, Wyatt & Costa, 1975). After evaporating the excess reagent with nitrogen, heptafluorobutyric anhydride (HFBA), 0.05 ml, was added and the sample heated at 50°C for 50 min. After heating, the solution was carefully reduced in volume using nitrogen and 0.2 ml of hexane were added. The hexane layer was then washed with 0.4 ml of 1.0 M phosphate buffer (pH 6.0) to remove any final traces of the excess HFBA. Immediately prior to injection into the g.c.-m.s. the solution was reduced in volume. The *p*- and *m*-HPAA isomers were estimated in a double focussing mass spectrometer [Vacuum Generator (VG) 70–70F] interfaced with a gas chromatograph (Hewlett-Packard 5710A). The column used to separate the isomers was a 50 m SCOT column coated with a mixture of 50 GS and OV–101 (Mandel Scientific, Rockwood, Ontario, Canada). The carrier gas employed was helium at a pressure of four pounds per square inch. Temperatures of the various zones were: injector 200°C, column 180°C isothermal and ion source 200°C. Further details of this procedure are to be found in the paper by Durden & Boulton (1981).

Drugs were dissolved in 0.9% w/v NaCl solution (saline) and injected subcutaneously. Haloperidol and sulpiride were dissolved in 50–100 μl of glacial acetic acid and then diluted 40 to 50 times with saline. (+)-Butaclamol hydrochloride and (−)-butaclamol hydrochloride were suspended in saline containing 2.2 mg ml^{-1} of Tween 80. Controls were injected

Table 1 Effect of the subcutaneous administration of (+)-butaclamol, (−)-butaclamol, haloperidol, sulpiride and molindone on the mouse striatal concentrations of *p*-HPAA and *m*-HPAA

Treatment	*Dose* (mg kg^{-1})	*Time* (h)	p-*HPAA* (ng g^{-1})	m-*HPAA* (ng g^{-1})
Vehicle controls	—	—	29 ± 3.6 (5)	13 ± 1.3 (5)
(+)-Butaclamol	1	2	71 ± 6.4 (5)**	33 ± 4.2 (5)*
(−)-Butaclamol	1	2	32 ± 1.2 (5)	12 ± 2.1 (5)
Vehicle controls	—	—	28 ± 1.3 (7)	8.8 ± 0.7 (7)
Haloperidol	0.2	2	28 ± 0.7 (5)	12 ± 2.0 (5)
Haloperidol	5	2	79 ± 7.5 (5)**	38 ± 4.4 (5)**
Sulpiride	50	3	37 ± 2.2 (5)*	23 ± 2.6 (5)**
Saline controls	—	—	32 ± 1.3 (29)	12 ± 1.3 (29)
Molindone	2	2	34 ± 2.9 (5)	13 ± 1.8 (5)
Molindone	100	2	230 ± 47.0 (5)**	180 ± 27.0 (5)**

Values are means ± s.e.mean (number of experiments in parentheses) in ng g^{-1} of fresh tissue. Increases are significant with respect to their corresponding vehicle-injected controls using Student's *t* test: *$P < 0.01$; **$P < 0.001$

with the corresponding vehicle solutions. The drugs were generously provided by the following laboratories: (+)-butaclamol hydrochloride and (−)-butaclamol hydrochloride, Ayerst Research Laboratories, Montreal, Quebec, Canada; chlorpromazine hydrochloride, Smith, Kline and French Laboratories, Philadelphia, Pennsylvania, USA; haloperidol, Janssen Pharmaceuticals, Beerse, Belgium; molindone hydrochloride, Endo Laboratories, Garden City, New York, USA; promethazine hydrochloride, E. Lilly and Co., Indianapolis, Indiana, USA and sulpiride, Delagrange International, Paris, France.

Results

The vehicle used to dissolve haloperidol produced a significant reduction in the mouse striatal concentrations of *p*- and *m*-HPAA (to 88 and 73%, respectively) with respect to the saline controls (Table 1). Therefore, the effects obtained after drug-injection were compared to the corresponding solvent-injected controls.

The subcutaneous administration of (+)-butaclamol (1 mg kg^{-1}) produced significant increases (to 245 and 254% of controls, respectively) in the mouse striatal concentration of *p*- or *m*-HPAA (Table 1); the effects were observed 2 h after the beginning of the treatment. In contrast, no significant changes were observed after the administration of (−)-butaclamol (Table 1). Marked increases in the concentration of both *p*- and *m*-HPAA were observed after the administration of haloperidol (5 mg kg^{-1}), sulpiride (50 mg kg^{-1}) or molindone (100 mg kg^{-1}) (Table 1). Lower doses of haloperidol (0.2 mg kg^{-1}) or molindone (2 mg kg^{-1}) produced no significant changes (Table 1).

The effect of the administration of a single large dose of chlorpromazine (20 mg kg^{-1}) upon *p*-HPAA or *m*-HPAA concentrations at various times is shown in Table 2. Significant increases in both acids were observed within 30 min after drug treatment and the concentrations reached 5 to 13 times their control levels (Table 2). The levels of *p*- and *m*-HPAA were still significantly higher than controls at 4 h after the beginning of the experiment (to 228 and 441% of controls respectively) (Table 2). By 8 h the concentrations of *p*- and *m*-HPAA were somewhat higher than controls though there was a clear tendency to return to these levels (Table 2). No significant changes in the mouse striatal concentration of *p*- or *m*-HPAA were observed 2 h after the administration of lower doses of chlorpromazine (2 mg kg^{-1}) or of promethazine (20 mg kg^{-1}) (Table 2).

Discussion

Antipsychotic drugs increase striatal dopamine (DA) metabolism (Carlsson & Lindqvist, 1963). Also they produce marked reductions in striatal *p*-tyramine, with no changes or with increases in *m*-tyramine (Juorio, 1977; 1979; 1980a). Administration of (+)-butaclamol, the clinically efficacious isomer, increased the concentration of both *p*- and *m*-HPAA while (−)-butaclamol was without effect (Table 1). It had already been shown that of the two isomers only (+)-butaclamol significantly elevated homovanillic acid concentrations (Lippmann, Pugsley & Merker, 1975) or changed the concentrations of *p* or *m*-HPAA in the caudate nucleus (Table 1). A large dose of chlorpromazine (20 mg kg^{-1}) administered to mice produced an increase in the striatal concentrations of *p*-HPAA and *m*-HPAA while a similar dose of promethazine, a phenothiazine lacking antipsychotic properties failed to affect the *p*-HPAA and *m*-HPAA in the mouse caudate nucleus (Table 2). Smaller doses of chlopromazine (2 mg kg^{-1}), that did not affect the *p*- or *m*-HPAA concentrations

Table 2 Effect of the subcutaneous administration of chlorpromazine or promethazine on the mouse striatal concentration of *p*-HPAA and *m*-HPAA

Treatment	*Dose* (mg kg^{-1})	*Time* (h)	p-*HPAA* (ng g^{-1})	m-*HPAA* (ng g^{-1})
Saline controls	—	—	32 ± 1.3 (29)	12 ± 1.3 (29)
Chlorpromazine	2	2	32 ± 2.7 (7)	14 ± 1.8 (7)
	20	0.5	150 ± 22.0 (5)**	130 ± 25.0 (5)**
	20	1	150 ± 17.0 (5)**	150 ± 25.0 (5)**
	20	2	97 ± 11.0 (17)**	70 ± 13.0 (17)**
	20	4	73 ± 8.4 (5)**	53 ± 20.0 (5)*
	20	8	46 ± 3.4 (5)**	19 ± 3.5 (5)
Promethazine	20	2	34 ± 3.0 (5)	8.7 ± 1.6 (5)

Values are means ± s.e.mean (number of experiments in parentheses) in ng g^{-1} of fresh tissue. Increases are significant with respect to saline controls using Student's *t* test: *$P < 0.05$; **$P < 0.001$

nevertheless decreased the concentrations of *p*-tyramine, (Juorio, 1977). Similarly, lower doses of haloperidol (0.2 mg kg^{-1}) or molindone (2 mg kg^{-1}) produced a reduction in mouse striatal *p*-tyramine and no change or an increase in *m*-tyramine (Juorio, 1977; 1979; 1980a) but did not affect either *p*- or *m*-HPAA (Table 1). The failure of the lower doses of the antipsychotic drugs to increase the tyramine acid metabolites while still decreasing the *p*-tyramine concentrations or increasing or not affecting *m*-tyramine suggests that at least two mechanisms could occur in this process. One of the mechanisms by which the lower doses of the antipsychotic drugs produce the decrease in *p*-tyramine may be by activation of tyrosine hydroxylase (Zivkovic, Guidotti & Costa, 1974). After tyrosine hydroxylase activation, the concentration of *p*-tyrosine becomes rate limiting (Sved & Fernstrom, 1981) thus reducing the amount of *p*-tyrosine available for decarboxylation (Westerink & Wirix, 1983); as a result the concentration of *p*-tyramine is reduced. This reduction in *p*-tyramine has been observed after the administration of a wide range of antipsychotic drugs (Juorio, 1977; 1979; 1980a). The fact that the inhibition of tyrosine hydroxylase by α-methyl-*p*-tyrosine (Spector, Sjoerdsma & Undenfriend, 1965) produced the opposite effects on brain tyramine concentrations (Juorio, 1979) further support this explanation. The other mechanism could be that the increased utilization and subsequent metabolism of dopamine (Andén *et al.*, 1964) could also involve the increased utilization and subsequent metabolism of *p*- and *m*-tyramine (Tables 1 and 2).

The administration of varying doses of molindone gives a possible dose differential effect. The effects of small doses of molindone (1–2 mg kg^{-1}) on mouse or rat striatal dopamine metabolism and tyramine concentration are quite similar to those produced by other antipsychotic drugs (O'Keeffe, Sharman & Vogt, 1970; Juorio, 1977; 1980a) and could be explained as being a result of dopamine receptor blockade and tyrosine hydroxylase activation. Larger doses of molindone (20–100 mg kg^{-1}) produced a reduction in dopamine metabolite concentration, increased *p*-tyramine and reduced *m*-tyramine (Juorio, 1980a). These effects are comparable to those of apomorphine, lergotrile or piribedil (Juorio, 1979), which possess dopamine receptor agonist activity. On the basis of these findings it was suggested that at lower doses molindone acts as a dopamine receptor blocker while at higher doses it possess a partial agonist effect (Juorio, 1980a). Recent work with the rat brain has confirmed the dose differential effects of molindone on dopamine metabolite concentration (Meller & Friedman, 1982). Interestingly, these authors found that small doses of molindone (2.5 mg kg^{-1}) produce tyrosine hydroxylase activation while high doses (40 mg kg^{-1}) produce tyrosine hydroxylase inhibition. Also, they found that molindone (10–50 mg kg^{-1}) produced marked monoamine oxidase inhibition (Meller & Friedman, 1982). In the mouse, the changes in *p*- and *m*-tyramine concentration after molindone administration (Juorio, 1980a) are compatible with dopamine receptor blockade observed with the low dose (2 mg kg^{-1}) or activation of dopamine receptors (partial agonist effect) observed with the higher dose of molindone (100 mg kg^{-1}). The inhibition of rat brain monoamine oxidase produced by the larger doses of molindone (Meller & Friedman, 1982) could explain the increases in rat striatal *m*-tyramine observed after treatment with 100 mg kg^{-1} of molindone (Juorio, 1980a).

The time course of the effect of the administration of chlorpromazine (20 mg kg^{-1}) shows that it causes a rapid release of *p*- or *m*-tyramine which are oxidatively deaminated by monoamine oxidase to form *p*- or *m*-HPAA. The site of this deamination has not been determined but it may be either within the presynaptic neurone, postsynaptic neurone or within any of these cellular components. The removal of *p*-HPAA from the caudate nucleus takes more than 8 h while that of *m*-HPAA takes at least 4 h (Table 2).

It has been claimed that sulpiride and molindone are more potent inhibitors of presynaptic than postsynaptic receptors (Costall, Hui & Naylor, 1980; Meller & Friedman, 1982). Both compounds also produce larger increases in caudate nucleus *m*-HPAA concentrations relative to *p*-HPAA concentrations, suggesting that *m*-HPAA (and *m*-tyramine) concentration changes may be indicative of presynaptic receptor changes.

The injections of relatively larger doses of antipsychotic drugs produces an increased firing of nigral dopamine neurones; this firing rate stimulation may be due to direct drug action or to blockade of presynaptic or postsynaptic dopamine receptors (Bunney, Walters, Roth & Aghajanian, 1973). The increased frequency of neuronal discharges releases large amounts of dopamine (Andén, Corrodi, Fuxe & Understedt, 1971) that in conjunction with blockade of the presynaptic and/or postsynaptic receptor sites leads to utilization of *p*-tyramine and *m*-tyramine possibly acting in a neuromodulatory role (Boulton, 1979). With the increased utilization of the tyramines, the concentrations of their respective metabolites *p*-HPAA and *m*-HPAA are also increased.

We wish to thank Dr A.A. Boulton and Dr P.L. Wood for their helpful discussions, Dr B.A. Davis for synthesizing the deuterated internal standards; Dr D.A. Durden and Dr C.W. Kazakoff for supervising the GC-MS determinations;

Mrs D. Choo for assistance with the animals and Ms S. Smith for performing the g.c.-m.s. determinations. The financial assistance of the Medical Research Council of Canada and Saskatchewan Health is gratefully acknowledged as is the award of a scholarship from the University of Saskatchewan to P.S. McQuade.

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(*Received June 10, 1983.*)