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EFFECTS OF MAZINDOL ON RAT BRAIN SYNAPTOSOMAL MONOAMINE UPTAKE

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Abstract—The effects of the anorectic drug mazindol on the uptake of [^{3}H]NA and [^{3}H]5HT by rat hypothalamic synaptosomes and the uptake of [^{3}H]DA by rat striatal synaptosomes were investigated. In *in vitro* studies drugs were added to the incubation medium. In *ex vivo* experiments drugs were injected i.p. at various times prior to death and synaptosomal [^{3}H] monoamine uptake subsequently determined. Two other anorectics (*d*-amphetamine and *dl*-fenfluramine) and two inhibitors of monoamine uptake (chlorimipramine and desipramine) were included for comparative purposes. Mazindol was a potent inhibitor of [^{3}H]NA and [^{3}H]DA uptake *in vitro* being approx. 0.5 times as potent as desipramine and *d*-amphetamine respectively. The abilities of mazindol, fenfluramine and desipramine to block the *in vitro* uptake of [^{3}H]5HT were comparable and all three drugs were appreciably less potent than chlorimipramine. Following 1 hr pretreatment, *d*-amphetamine was the most potent of the five drugs at inhibiting synaptosomal [^{3}H]NA and [^{3}H]DA uptake. Mazindol was approx. 2.5 times more potent than desipramine at blocking [^{3}H]NA uptake. In contrast to the other drugs, pretreatment with large doses of mazindol had essentially no effect on hypothalamic synaptosomal [^{3}H]5HT uptake. Results of *ex vivo* studies thus confirm *in vivo* findings that mazindol is a selective inhibitor of rat brain catecholamine uptake.

Mazindol is an effective anorectic agent in both animals and humans [1, 2]. Previous studies from this and other laboratories have shown that mazindol is a potent inhibitor of noradrenaline (NA) uptake by rat brain *in vivo* [3–5]. *In vivo* dopamine (DA) uptake by rat brain is also blocked by the drug though to a lesser extent than NA [3, 5]. In contrast to its ability to inhibit catecholamine uptake, mazindol was found to be essentially devoid of effect on the uptake of 5-hydroxytryptamine (5HT) by rat brain *in vivo* as indicated by the inability of pretreatment with large doses of the drug to block the fall in brain 5HT content following the i.p. injection of *p*-chloroamphetamine [3]. The objective of this study was to investigate the effects of mazindol on the uptake of NA, DA and 5HT by synaptosome rich homogenates obtained from selected regions of rat brain. Two experimental approaches were used. In the first, drugs were directly added to the incubation medium at the start of the preincubation period (*in vitro* experiments). In the second, rats were injected i.p. with the drug under study at various times prior to death and synaptosomal uptake subsequently determined (*ex vivo* experiments). Two other anorectics (*d*-amphetamine and *dl*-fenfluramine) and two inhibitors of monoamine uptake (desipramine and chlorimipramine) were included for comparative purposes. A preliminary account of some of the findings has been published in abstract form [6].

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MATERIALS AND METHODS

Materials. The following compounds were generously donated: mazindol (Sandoz), *d*-amphetamine sulphate and SKF-525A (Smith Kline and French), *dl*-fenfluramine hydrochloride (Servier), chlorimipramine hydrochloride and desipramine hydrochloride (Geigy). [G-^{3}H] 5-hydroxytryptamine creatinine sulphate (14.0 Ci/mmole), L-[7-^{3}H] noradrenaline (5.8 Ci/mmole) and [ethylamine-1,2-^{3}H] dopamine hydrochloride (8.5 Ci/mmole) were purchased from the Radiochemical Centre, Amersham, England.

Methods. Male Wistar rats (ALA/CFHB strain) weighing 180–250 g were used. The uptake of [^{3}H]DA into synaptosome rich homogenates of corpus striatum and the uptake of [^{3}H]NA and [^{3}H]5HT into synaptosome rich homogenates of hypothalamus were measured as described in detail elsewhere [7]. Briefly the weighed tissue was homogenized by a motor driven Teflon pestle in a glass homogenizer to yield a uniform homogenate. Following centrifugation at 1000 *g* for 10 min at 4° the resultant supernatant was decanted and stirred to give a uniform suspension. An aliquot of the suspension was preincubated in a modified Krebs-bicarbonate buffer for 10 min at 37° under an atmosphere 59% O_2–5% CO_2 in a Dubnoff metabolic shaker after which [^{3}H] monoamine was added (final concentration of 2.6×10^{-8} M^{-6} M). The composition (mmole/l.) of the buffer solution was: NaCl, 118.5; KCl, 4.74; $MgSO_4$. $7H_2O$, 1.19; $CaCl_2$, $6H_2O$, 1.28; KH_2PO_4, 1.19; $NaHCO_3$, 25.0; glucose, 11.0; pargyline, 0.15; ascorbic acid, 0.11. Incubation was continued for 10, 5 or 2 min for [^{3}H]NA, [^{3}H]5HT or [^{3}H]DA respectively. In *in vitro* experiments drugs were added at the start of

the preincubation period. Incubation was terminated by the addition of 5 ml ice-cold saline (0.9 per cent) and then standing the beakers in ice for 10 min. The homogenate was separated from the medium by filtration under vacuum. After a further rinse with 5 ml of ice-cold saline the filter disc was placed in a counting vial and 15 ml Bray's scintillant added. Samples were counted in a Nuclear Chicago Isocap 300 scintillation counter. The concentration of monoamine taken up was calculated in terms of dpm/g original tissue divided by dpm/ml medium and was corrected for non-specific binding by subtracting the amount taken up at 0°.

Results of *in vitro* experiments were expressed as the IC_{50} value which is defined as the molar concentration of drug required to inhibit uptake by 50 per cent. IC_{50} values were obtained from log dose/probit inhibition graphs at four concentrations of drug in quadruplicate. Results of *ex vivo* experiments were expressed either as per cent inhibition of monoamine uptake or as the ED_{50} value which is defined as the dose of drug (mg/kg) required to inhibit uptake by 50 per cent and was obtained from per cent uptake inhibition/log dose response curves constructed by the method of least squares: Each log dose/response curve had at least three points and each point was the mean of at least four determinations. In all *ex vivo* experiments doses refer to the free base and except for mazindol which was suspended in 5% v/v mulgofen in distilled water drugs were dissolved in saline.

Table 1. *In vitro* inhibition of [^{3}H] monoamine uptake

Drug	IC_{50} (M) [^{3}H]5HT	[^{3}H]NA	[^{3}H]DA
Mazindol	1.9×10^{-7}	5.7×10^{-8}	5.3×10^{-7}
Desipramine	3.1×10^{-7}	3.2×10^{-8}	8.0×10^{-6}
Fenfluramine	3.7×10^{-7}	2.3×10^{-7}	1.1×10^{-5}
Chlorimipramine	7.9×10^{-9}	1.1×10^{-7}	2.2×10^{-6}
d-Amphetamine	2.1×10^{-5}	4.7×10^{-7}	2.8×10^{-7}

Rat striatal synaptosomes were used for [^{3}H]DA studies and hypothalamic synaptosomes for [^{3}H]NA and [^{3}H]5HT studies. Drugs were preincubated with synaptosomes for 10 min after which [^{3}H] monoamine (final concentration 2.6×10^{-8} M) was added and incubation continued for 10, 5 or 2 min for [^{3}H]NA, [^{3}H]5HT or [^{3}H]DA respectively. IC_{50} values, i.e. concentration of drug required to produce 50 per cent inhibition of [^{3}H] monoamine uptake, were obtained from log dose/inhibition probit graphs at four concentrations of drug in quadruplicate.

RESULTS

In vitro *experiments.* The effects of the agents studied for *in vitro* inhibition of [^{3}H] monoamine uptake are summarized in Table 1. Of the compounds studied for inhibition of [^{3}H]5HT uptake by rat hypothalamic synaptosomes, mazindol, desipramine and fenfluramine were of comparable potency and all three compounds were less potent than chlorimipramine. Mazindol and desipramine were the most potent of the compounds studied for inhibition of [^{3}H]NA uptake. Fenfluramine, desipramine and chlorimipramine were appreci-

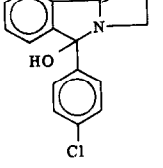

Fig. 1. Structure of mazindol.

Table 2. Effect of 1 hr pretreatment on the uptake of [^{3}H]5HT into rat hypothalamic synaptosomes

Drug	Dose (mg/kg i.p.)	Per cent inhibition	ED_{50} (mg/kg)	95 per cent confidence limits
Chlorimipramine	10	41.5 ± 0.3		
	20	56.3 ± 0.9	14.5	(13.4–15.6)
	40	82.6 ± 0.7		
d-Amphetamine	3.75	8.6 ± 5.5		
	7.5	32.6 ± 6.3	21.3	(18.2–26.0)
	15	42.4 ± 1.8		
	30	56.2 ± 4.0		
Fenfluramine	10	36.6 ± 3.4		
	20	46.4 ± 1.1	24.6	(20.2–29.7)
	40	51.6 ± 0.3		
	60	70.0 ± 1.6		
Desipramine	30	13.2 ± 4.0		
	60	49.1 ± 1.0	>60	
Mazindol	30	4.2 ± 4.0		
	60	12.0 ± 4.3	>60	

Hypothalamic synaptosomes from rats injected i.p. 1 hr prior to killing were incubated for 5 min in [^{3}H]5HT (2.6×10^{-8} M). Each result, expressed as per cent inhibition, is the mean ±S.E.M. of four determinations. Doses of drugs (mg/kg) which cause 50 per cent inhibition of uptake (ED_{50}) and 95 per cent confidence limits were calculated by the method of least squares regression.

ably less potent than *d*-amphetamine and mazindol at blocking striatal [^{3}H]DA uptake.

Ex vivo *experiments.* Chlorimipramine was the most potent of the drugs studied for inhibition of [^{3}H]5HT uptake by rat hypothalamic synaptosomes following 1 hr pretreatment (Table 2). Mazindol, in contrast to the other drugs studied, was essentially devoid of effect on [^{3}H]5HT uptake. The ability of mazindol (60 mg/kg, i.p.) to block hypothalamic synaptosomal [^{3}H]5HT uptake was increased from 11.6 ± 4.1 to 34.9 ± 9.3 per cent (n = 6) following 1 hr pretreatment with SKF-525A (40 mg/kg, i.p.). However, it is to be noted that this inhibition of uptake is still appreciably less than that of the other drugs studied. Although mazindol was virtually devoid of effect on hypothalamic synaptosomal [^{3}H]5HT uptake 1 hr after injection it is feasible that a drug-induced inhibition of [^{3}H]5HT uptake could be observed at other times. The effects of 1, 2 and 4 hr i.p. pretreatment with mazindol (30 and 60 mg/kg) and the approx. ED_{50} values of chlorimipramine (15 mg/kg), fenfluramine (30 mg/kg) and desipramine (60 mg/kg) were studied. At no time did mazindol, in contrast to the other drugs studied, block [^{3}H]5HT uptake by approximately 25 per cent or more (Table 3).

d-Amphetamine was an extremely potent inhibitor of [^{3}H]DA uptake by rat striatal synaptosomes following 1 hr pretreatment (Table 4). On the basis of ED_{50} values, *d*-amphetamine was approximately 18 times more potent than mazindol. Fenfluramine, desipramine and chlorimipramine were appreciably less potent than mazindol at blocking striatal [^{3}H]DA uptake 1 hr after injection.

As in the *ex vivo* DA studies, *d*-amphetamine was the most potent of the drugs studied for inhibition of [^{3}H]NA uptake by hypothalamic synaptosomes following 1 hr pretreatment. Mazindol was also a potent inhibitor having an i.p. ED_{50} value of 8.1 mg/kg (Table 5). At 2 and 4 hr after mazindol (8 mg/kg, i.p.) the uptake of [^{3}H]NA by rat hypothalamic synaptosomes was blocked by 56.3 ± 3.6 and 34.1 ± 1.3 per cent respectively. Inhibition of synaptosomal [^{3}H]NA uptake at 2 and 4 hr after desipramine (20 mg/kg, i.p.) was 61.3 ± 3.8 and 60.5 ± 5.4 per cent respectively (n = 6).

DISCUSSION

The effects of mazindol on *in vitro* [^{3}H] monoamine uptake observed in this study agree with the findings of others. For example, mazindol rivals *d*-fenfluramine and desipramine at inhibiting in *in vitro* uptake of 5HT by both rat and guinea pig platelets [8, 9] and by rat striatal synaptosomes [10]. Moreover, the observation that mazindol is a potent inhibitor of synaptosomal [^{3}H]NA and [^{3}H]DA uptake confirms the observations of others [10–13].

Results of experiments investigating drug effects on the *ex vivo* uptake of [^{3}H]NA and [^{3}H]DA are generally in qualitative agreement with *in vitro* findings. For example, mazindol rivals desipramine at blocking hypothalamic synaptosomal [^{3}H]NA

Table 3. Blockade of [^{3}H]5HT uptake at various times after injection

Drug	Dose (mg/kg, i.p.)	Per cent inhibition Time after injection (hr) 1	2	4
Mazindol	30	4.2 ± 4.0	11.2 ± 2.6	14.4 ± 1.5
	60	12.0 ± 4.3	22.5 ± 4.5	18.5 ± 3.8
Chlorimipramine	15	68.4 ± 2.7	42.8 ± 4.5	32.5 ± 1.6
Fenfluramine	30	46.8 ± 0.7	60.6 ± 2.0	75.3 ± 0.8
Desipramine	60	41.9 ± 1.0	32.8 ± 2.8	31.6 ± 1.4

Rats were killed at times stated after injection and synaptosomal [^{3}H]5HT uptake determined. For remainder of legend, see Table 2.

Table 4. Effect of 1 hr pretreatment on the uptake of [^{3}H]DA into rat striatal synaptosomes

Drug	Dose (mg/kg, i.p.)	Per cent inhibition	ED_{50} (mg/kg)	95 Per cent confidence limits
d-Amphetamine	0.5	21.8 ± 4.8		
	1	37.0 ± 0.5		
	2	43.8 ± 2.3	2.1	(1.7–2.5)
	5	69.3 ± 1.4		
Mazindol	15	21.1 ± 1.6		
	30	35.0 ± 5.1	39.0	(34.7–44.9)
	60	62.9 ± 3.2		
Fenfluramine	60	35.4 ± 4.1	>60	
Chlorimipramine	60	28.4 ± 5.4	>60	
Desipramine	60	24.3 ± 6.0	>60	

Striatal synaptosomes from rats injected i.p. 1 hr prior to killing were incubated for 2 min in [^{3}H]DA (2.6×10^{-8} M). For remainder of legend, see Table 2.

Table 5. Effect of 1 hr pretreatment on the uptake of [^{3}H]NA into rat hypothalamic synaptosomes

Drug	ED_{50} (mg/kg, i.p.)	95 per cent confidence limits
d-Amphetamine	3.1	2.3–4.1
Mazindol	8.1	3.9–10.5
Desipramine	20.4	4.2–38.2
Fenfluramine	20.9	19.2–22.7
Chlorimipramine	26.5	21.5–33.7

Hypothalamic synaptosomes from rats injected i.p. 1 hr prior to killing were incubated for 10 min in [^{3}H]NA (2.6×10^{-8} M). For remainder of legend, see Table 2.

uptake both *in vitro* and *ex vivo* and the weak inhibitory effects of fenfluramine on both *in vitro* and *ex vivo* [^{3}H]DA uptake agrees with the data of Kannengiesser *et al.* [14]. However, anomalies exist. For example, mazindol and *d*-amphetamine are of comparable potency at blocking striatal synaptosomal [^{3}H]DA uptake *in vitro* whereas *d*-amphetamine is appreciably more potent *ex vivo*, a finding which has also been reported by Carruba *et al.* [12]. Regarding the uptake of [^{3}H]NA by rat hypothalamic synaptosomes mazindol and desipramine are more potent than *d*-amphetamine *in vitro* whereas the converse is the case in *ex vivo* studies. The enhanced effect of *d*-amphetamine after *in vivo* administration is probably due to its rapid and easy passage into the brain [15]. The lack of effect of 1 hr pretreatment with large doses of mazindol on the *ex vivo* uptake of [^{3}H]5HT by hypothalamic synaptosomes was unexpected in light of *in vitro* findings which revealed that mazindol, fenfluramine and desipramine were of comparable potency. Both fenfluramine and desipramine blocked the *ex vivo* uptake of [^{3}H]5HT and the long duration of uptake inhibition by fenfluramine is in agreement with the data of others [14]. Why mazindol is active in blocking [^{3}H]5HT uptake *in vitro* but not *ex vivo* is not readily apparent. One possibility is that mazindol does not attain and maintain a sufficiently high concentration in the brain to compete with 5HT for the amine carrier located at the axonal membrane. However, this is unlikely since mazindol inhibits catecholamine uptake both *in vitro* and *ex vivo*. Furthermore, the *ex vivo* uptake of [^{3}H]NA is markedly antagonized at 2 and 4 hr after the injection of the drug at a dose appreciably lower than that having essentially no effect on *ex vivo* [^{3}H]5HT uptake (8 mg/kg *vs* 60 mg/kg). Mazindol is extensively metabolized and a number of metabolites have been identified in rat urine [16]. Hence it is possible that mazindol is metabolized to a metabolite which is devoid of effect on [^{3}H]5HT uptake but blocks [^{3}H]NA uptake. To test this hypothesis rats were pretreated with the liver microsomal enzyme inhibitor SKF-525A [17] using a dosage and time schedule which has been shown to appreciably elevate *d*-amphetamine levels in the rat brain [18]. Although the ability of mazindol to block hypothalamic synaptosomal [^{3}H]5HT uptake at 1 hr after injection was enhanced by SKF-525A pretreatment the enhanced block was less than that following 1 hr pretreatment with either fenfluramine or desipramine.

The ability of mazindol to block the *ex vivo* uptake of NA and DA is in agreement with results of studies in which inhibition of NA and DA uptake *in vivo* was assessed using indirect methods [3, 5, 19]. There is data suggesting that the *in vivo* uptake of 5HT by rat brain is inhibited by mazindol. For example, Carruba *et al.* [20] observed that mazindol injected after *p*-chloroamphetamine administration prevented the fall in rat brain 5HT content induced by the latter agent. In addition, mazindol pretreatment partially blocked the fall in rat brain 5HT levels following fenfluramine administration [5]. However, in other studies mazindol was observed to have no effect on the *in vivo* uptake of 5HT as assessed by the effect of drug pretreatment on the ability of *p*-chloroamphetamine to lower the 5HT content of rat [3] and mouse [19] brain. The lack of effect of mazindol on the *ex vivo* uptake of 5HT observed in this study is in agreement with the findings of Sugrue *et al.* [3] and Fuller *et al.* [19]. Germane to the concept of mazindol having little or no effect on 5HT uptake *in vivo* is the lack of effect of the drug on rat brain 5HT turnover [3, 21]. In contrast to mazindol compounds which inhibit the *in vivo* uptake of 5HT, e.g. chlorimipramine [22], fluoxetine [23], Org 6582 [24] and citalopram [25], all decrease the turnover of the monoamine in the rat brain, a phenomenon attributed to receptor mediated feedback regulation.

Mazindol differs from fenfluramine in that it is generally agreed that the anorectic effect of mazindol is unlikely to be mediated via central 5HT systems [3, 5, 11, 26]. Mazindol induced anorexia does not appear to be due to a direct effect on central catecholaminergic receptors since the ability of the drug to decrease rat food intake in antagonized by depleting the brain of its catecholamine content [5, 27, 28]. The behavioural and anorectic effects of mazindol and *d*-amphetamine have been compared in a number of studies and as a result of these investigations DA has been implicated in the anorectic effect of mazindol [11, 12, 28]. However, mazindol is not a potent inhibitor of DA uptake *in vivo* [3, 5] and *ex vivo* and the ability of mazindol to release DA *in vivo* [3, 26] and *in vitro* [12, 13, 29] is much less than that of *d*-amphetamine. Mazindol has essentially no effect on NA release [4, 13] and the possibility that the potent inhibitory effect of the drug on NA uptake is implicated in mazindol induced anorexia warrants consideration.

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