Effects of the optical isomers of 2-amino-3-fluoro-1-phenylpropane (monofluoroamphetamine) hydrochloride on uptake and release of dopamine in rat striatum in vitro[1]

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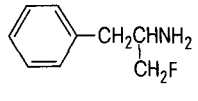
Summary. The (+)- and (−)-isomers of the monofluorinated amphetamine 2-amino-3-fluoro-1-phenylpropane were prepared and compared for their ability to stimulate release and to inhibit reuptake of radiolabelled dopamine from rat striatal tissue in vitro. The (+)-isomer was much stronger than the (−)-isomer in both cases.

Replacement of one or more hydrogens in the alkyl side chain of amphetamines can have dramatic effects on the ability of these drugs to penetrate the brain, on their metabolism, and on their ability to interact with enzymes and neurotransmitter amines[3–5]. Although fluorine is similar in size to hydrogen, the electron-withdrawing properties of fluorine will reduce the basicity of the adjacent nitrogen and, if this reduction in basicity is marked, the degree of ionization of the drug at physiological pH will be altered. This, combined with the increased lipophilicity imparted by fluorine can affect significantly the metabolism of amphetamines and their ability to interact with various biological membranes in the nervous system.

We have prepared monofluorinated analogues of (±)-amphetamine and (±)-*p*-chloroamphetamine (pCA) in which a single hydrogen atom of the *α*-methyl substituent was replaced with a fluorine. These compounds have pKa values approximately 1.65 units lower than the parent amphetamines and are significantly weaker than amphetamine and pCA in stimulating release of the neurotransmitters dopamine (DA) and 5-hydroxytryptamine (5HT) respectively from rat brain striatal tissue in vitro[7]. As an extension of this work, we also, prepared the pure optical isomers of monofluorinated amphetamine (2-amino-3-fluoro-1-phenylpropane, I) and compared their ability to inhibit uptake of and to release DA from rat brain striatal tissue. This study is described in the present report.

Methods. The optical isomers, (+)- and (−)-2-amino-3-fluoro-1-phenylpropane hydrochloride (**Ia**, $[\alpha]_D^{22} = +20.42°$, and **Ib**, $[\alpha]_D^{22} = -19.89°$, respectively) were synthesized from (+)- or (−)-phenylalanine via aziridine intermediates by a novel synthetic procedure for the preparation of *α*-(monofluoromethyl)amines[6]. Structures were confirmed by gas chromatography-mass spectrometry, NMR spectroscopy, elemental analysis and polarimetry.

Uptake and release were studied in prisms (0.1 mm × 0.1 mm × approximately 2 mm) of rat brain striatum using previously described procedures[8,9]. The concentration of ^{3}H-DA employed in these experiments was 0.02 μM, and nialamide (12.5 μM) was included in the incubation and superfusion media to inhibit monoamine oxidase. In uptake experiments, the prisms (final concentration: 5 mg/5 ml) were preincubated at 37°C for 15 min in modified Krebs' medium, the drug and radiolabelled DA were added, and incubation was continued for a further 7 min. At this time, the tissue was isolated, under light vacuum, on circular filter papers on solid supports, and washed rapidly with 2 × 5 ml of medium. The filters were then removed from their supports, and total radioactivity was measured in a liquid scintillation counter. In the release studies, the prisms were preincubated as described above, the labelled DA was added, incubation was continued for 7 min, and the tissue was isolated on filter papers supported in thermostatically jacketed superfusion chambers. After washing rapidly with 2 × 5 ml of medium, the tissue in parallel superfusion chambers was superfused in the presence or absence of drugs, as described in figure 2.



Ia: (+) isomer
Ib: (−) isomer

Fig. 1. Structure of 2-amino-3-fluoro-1-phenylpropane.

Results and discussion. Figure 2 illustrates that the (+)-isomer is a much stronger releaser of ^{3}H-DA than is the (−)-isomer. The mean amount of ^{3}H-DA released above control values by the former is approximately 5 times the amount released by the latter. A similar situation is true for inhibition of DA uptake, with the IC_{50} value for **Ia** being 0.82 μM and for **Ib** 3.8 μM. The relative importance of inhibition of reuptake and facilitation of release in the mode of action of amphetamine at nerve terminals and the failure of some techniques to differentiate between these 2 processes has been the source of considerable debate[8,10,11]. The superfusion technique used in the study reported here eliminates reuptake, and the release observed should be true release[8], but it is conceivable that the reduction in tissue ^{3}H-DA concentration in the uptake experiments could be due, at least in part, to releasing effects of the amphetamines occurring during the incubation[10,11].

In a previous publication, we reported that (±)-2-amino-3-fluoro-1-phenylpropane was weaker than (±)-amphetamine in stimulating release of ^{3}H-DA from rat striatal tissue in vitro[7]. However, this monofluorinated compound still retained considerable releasing activity, and results of

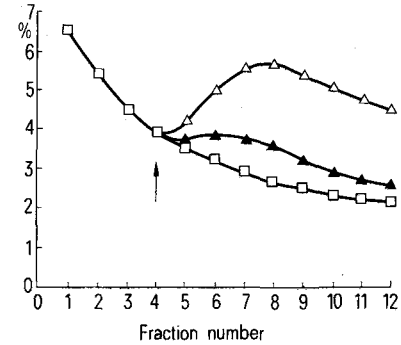


Fig. 2. Effects of (+)- and (−)-2-amino-3-fluoro-1-phenylpropane (1.0 μM) on release of ^{3}H-dopamine from prisms of rat striatum: □, control values; △, (+)-2-amino-3-fluoro-1-phenylpropane added; ▲, (−)-2-amino-3-fluoro-1-phenylpropane added. Samples of tissue preloaded with ^{3}H-dopamine were superfused with incubation medium at 0.5 ml/min and serial 1-min fractions were collected. After 4 min, the medium was replaced with further control medium in the case of the controls or with medium containing the appropriate drug. The arrow marks the fraction at which the drugs were added. The results are the means of 10 experiments run on separate days in parallel superfusion chambers. The values on the ordinate represent the percentage of total radioactivity (amount in fractions plus amount on filter at end of superfusion period) present in each collected fraction.

the present study show that, as with amphetamine[12-14], the ability to stimulate release and inhibit uptake of DA resides primarily in the (+)-isomer.

It is concluded that, in vitro, the optical isomers of monofluorinated amphetamine behave in much the same way as those of the parent amphetamine with regard to interaction with transport of the neurotransmitter DA. Studies are now underway to investigate the effects of these new compounds in vivo on brain DA and DA-mediated behaviour.

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Tolerance to, and symmetrical cross-tolerance between, cannabinol and Δ^9-tetrahydrocannabinol[1]

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Summary. Tolerance to, and symmetrical cross-tolerance between, cannabinol and Δ^9-tetrahydrocannabinol (THC) occurs in rabbits which uniquely exhibit behavioral convulsions with THC.

It is well established that Δ^9-tetrahydrocannabinol (THC) is the major psychoactive ingredient in marijuana[2], and that, following chronic administration, tolerance to many of its effects occur in both laboratory animals[3-5] and humans[6]. However, the activity in humans of acute cannabinol (CBN), another major constituent of marijuana, is equivocal[7-10]. Although data on chronic effects are lacking, acute CBN is active, albeit much less potent than THC, in laboratory animals[11-13], including tetrahydrocannabinol seizure susceptible (THC-SS) rabbits[14]. The THC-SS rabbits are unique in that they exhibit a selective response following low dose, i.v., injection of THC but not following noncannabinoid psychoactive drugs[14]. In the present study, the chronic effects of CBN and THC, and their subsequent interactive effects in THC-SS rabbits were investigated.

Materials and methods. Rabbits, weighing 1.3–3.8 kg, selected from our closed stock of THC-SS rabbits, were implanted with a cannula in the external jugular vein under pentobarbital anesthesia so that drugs could be repeatedly and conveniently administered. 6 rabbits were studied since the THC-SS rabbits, bred exclusively in our closed colony (Uaz:NZW), were limited in number. 7 days were allowed for recovery before testing. THC and CBN were prepared in a vehicle of a 10% polysorbate (Tween)-80 and 90% distilled water solution. During testing, individual rabbits were observed, for behavioral convulsions, through a 1-way

Development of behavioral convulsant tolerance to THC (A) and CBN (B) and cross-tolerance between the 2 cannabinoids (A and B) in THC seizure susceptible rabbits[a]

Rabbit identification number	Days of drug administration 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
A																					
358	0.1	0.1	0.1	0.1	0.1	0.1*	0.5	0.5	0.5	0.5	0.5	0.5	0.5*	15* CBN							0.1
351	0.1	0.1*	0.5	0.5	0.5	0.5	0.5	0.5	0.5*	15* CBN							0.1				
354	0.1	0.1	0.1*	0.5	0.5*	15* CBN							0.1*		0.5						
B																					
319	15	15	15*	20	20	20	20	20	20	20	20*	0.1* THC							15		
320	15	15	15	15	15	15*	20	20	20	20*	0.1* THC							15			
321	15	15	15	15*	20*	25*	30	30*	0.1* THC							15					

[a] Asterisks indicate no convulsion occurred; convulsions occurred on all other days of i.v. CBN (15, 20, 25 or 30 mg/kg) and THC (0.1, 0.5 mg/kg) administration.