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EFFECTS OF FLUORO-DOPAMINES ON DOPAMINE RECEPTORS (D_1, D_2, D_3 SITES)

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Abstract—The ring-fluorinated compounds, 2-, 5- and 6-fluoro-derivatives of dopamine, were tested on three dopamine-sensitive receptor sites in striatal tissue of rat and calf brain. Although the D_1 site (dopamine-sensitive adenylate cyclase) was stimulated by micromolar concentrations of both dopamine and the fluoro-dopamines, the latter were considerably weaker. The D_2 receptor, as measured by the binding of [^{3}H]spiperone, was occupied by similar concentrations of dopamine and the fluoro-dopamines, with IC_{50} values ranging from 17,000 to 63,000 nM. The D_3 receptor site, as measured by the high-affinity binding of [^{3}H]dopamine, was also occupied by similar concentrations of these compounds. Since it is primarily the D_2 receptor that is related to various dopaminergic behaviours, the similar potencies of dopamine and the fluoro-dopamines at this receptor site further validate the use of ^{18}F-compounds to study dopamine function by non-invasive means.

In order to study the intracerebral metabolism of dopamine by a non-invasive method, we have synthesized [^{18}F]-5-fluoro-DOPA, a positron-emitting analogue of DOPA and a precursor of 5-fluoro-dopamine [1]. Many of the biological properties of 5-fluoro-dopamine and 5-fluoro-DOPA are similar to those of dopamine and DOPA [2, 3]. The purpose of the present study was to determine whether the ring-fluorinated dopamines were also similar to dopamine in acting directly on the D_1, D_2 and D_3 dopamine-sensitive receptor sites *in vitro*.

The D-1 dopamine receptor had been originally designated as that dopamine receptor which is linked to dopamine-sensitive adenylate cyclase, and the D-2 receptor as that dopamine receptor which is not linked to this enzyme [4]. It has recently become necessary to modify these definitions slightly for several reasons.

First, there is no general agreement as to whether the mammotrophs in the anterior pituitary contain dopamine-stimulated adenylate cyclase. For example, Schmidt and Hill [5] could not detect any dopamine-stimulated adenylate cyclase in pituitary tissue; Ahn *et al.* [6], however, reported that they first found it necessary to preincubate the pituitary gland for 2–3 hr before homogenizing the tissue and detecting dopamine-stimulated adenylate cyclase. More recently, Thorner *et al.* [7] reported that dopamine had no effect on adenylate cyclase activity in homogenates of anterior pituitary glands or on the levels of cyclic AMP in the eluate from columns of anterior pituitary cells. Thus, although the weight of the evidence suggests the absence of dopamine-stimulated adenylate cyclase in the anterior pituitary cells, there is also evidence that dopamine can *inhibit* adenylate cyclase in prolactin adenomas [8] and in the intermediate lobe of the pituitary [9, 10]. Thus, the D-1, D-2 terminology of Kebabian and Calne [4] does not conveniently apply to the pituitary.

A second difficulty with the D-1, D-2 terminology of Kebabian and Calne [4] is that there is no simple way to determine the proportion of dopamine receptors which are linked to adenylate cyclase and of those which are not so linked. A third difficulty with the D-1, D-2 terminology of Kebabian and Calne [4] is that there is no relation between the biological potencies (for producing behavioural effects) of dopaminergic agonists or antagonists and their potencies on dopamine-sensitive adenylate cyclase [11, 12].

The current convenient use of the term "D_1", therefore, simply defines, indicates, or serves as an abbreviation for dopamine-stimulated adenylate cyclase; this D_1 enzyme is further *defined* or *characterized* by the properties of being stimulated by micromolar concentrations of dopamine [4] and being antagonized by micromolar concentrations of most neuroleptics [11].

The common current definition of the D_2 type of dopamine receptor is that dopamine receptor (labelled by any ^{3}H-ligand) which is blocked by nanomolar concentrations of neuroleptics but is occupied by micromolar concentrations of dopamine. A more complete definition and analysis of

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this receptor is given by Seeman [12] and also by Costall and Naylor [13].

The definition of the D_3 receptor site is that dopaminergic site (labelled by any ^{3}H-ligand) which is occupied by nonomolar concentrations (1–10 nM) of dopamine but is antagonized or occupied by micromolar concentrations of antagonists (i.e. neuroleptics) [14, 15].

When fluorine replaces a hydrogen atom in the aromatic ring of dopamine, the size of the molecule is not appreciably altered [16]. Since this fluoride substitution can exert a powerful ionising effect on the hydroxyl group of phenol [17], it was essential to examine different fluoro-dopamines (e.g. 2-, 5- and 6-fluoro-dopamine) to determine whether this factor seriously affects the interaction of dopamine with the three types of receptor sites. This present study revealed that these fluoro-dopamines had potencies generally similar to that of dopamine on the D_1, D_2 and D_3 receptor sites.

MATERIALS AND METHODS

Fluoro-dopamines. The syntheses of the 2-, 5- and 6-fluoro-derivatives of dopamine were carried out by the method of Kirk [18]. The pK_{OH} of each amine was determined spectrophotometrically by the method of Martin [19].

Measurement of dopamine-sensitive adenylate cyclase activity. Dopamine-sensitive adenylate cyclase activity (D_1 receptor sites) was measured in homogenates of rat striatum, using the method of Mishra *et al.* [20]. Briefly, the fresh striata were homogenized (all-glass homogenizer) in 2 mM Tris–maleate buffer (pH 7.4) containing 0.8 mM ethyleneglycol-bis-(aminoethylether)tetra-acetate (EGTA) (1 g tissue/75 vol. medium). Triplicate aliquots of 50 μl of homogenate were incubated at 30° (2.5 min) in a final volume of 200 μl in medium containing 80 mM Tris–maleate (pH 7.4), 10 mM theophylline, 2 mM $MgSO_4$, 0.5 mM ATP, and the test substance. The reaction was terminated by placing the assay tubes in a boiling water bath for 2.5 min. Particulate matter was removed by low-speed centrifugation, and aliquots of the supernatant fluids were assayed for cyclic AMP.

Binding of [^{3}H]spiperone to D_2 receptors. The binding of [^{3}H]spiperone to D_2 dopamine receptors was done using homogenates of calf caudate nucleus. There are no significant differences between the rat striatum, the human caudate nucleus and the calf caudate nucleus when one compares the concentrations (IC_{50} values) of dopamine and other drugs which inhibit by 50 per cent the specific binding of [^{3}H]spiperone to these tissues [14, 15, 21]. As detailed elsewhere [22], crude homogenates of calf caudate nucleus were resuspended in 10 vol. of ice-cold buffer [15 mM Tris–HCl, pH 7.4, 5 mM Na_2 EDTA, 1.1 mM (0.02%) ascorbic acid, and 12.5 μM nialamide] and stored frozen at −20° until used. Before assaying, the sample was thawed and centrifuged (15 min at 44,000 *g*) at 4°. The supernatant was discarded, and the pellet was resuspended in 5 ml buffer. The tissue was further homogenized using a Brinkmann Polytron (PT-10) at a setting of 7.0 (full scale = 10) for 20 sec. The final protein concentration of the suspension was 0.75 mg/ml. Each assay tube received 200 μl of buffer containing the drug being tested, 200 μl of [^{3}H]spiperone (final concentration was 0.07–0.1 nM; 23-26 Ci/mmole; New England Nuclear Corp., Boston, MA), and 200 μl of calf caudate homogenate. After incubation at room temperature (20–21°) for 30 min, 0.5 ml of the mixture was filtered through Whatman glass fibre filters (GF/B) under vacuum. The filters were rinsed with 10 ml of buffer and then placed in liquid scintillation vials; 8 ml of Aquasol (New England Nuclear Corp.) was added. The vials were stored at 4° for at least 6 hr to permit the filters to become translucent. Assays were done in quadruplicate. The specific binding of [^{3}H]spiperone was defined as that amount of [^{3}H]spiperone binding which could be inhibited by 100 nM spiperone.

Binding of [^{3}H]dopamine to D_3 dopaminergic receptor sites. The binding of [^{3}H]dopamine to D_3-type dopaminergic receptor sites was measured as previously described [14, 15], using calf caudate nucleus tissue. In our experience the IC_{50} values of various drugs on the binding of [^{3}H]dopamine are the same for rat striatum, human caudate nucleus and calf caudate nucleus [14, 15, 23]. The initial preparation of the tissue was the same as that described in the previous section for [^{3}H]spiperone binding, except that the crude homogenate was centrifuged and resuspended (in 15 vol.) four times (to remove endogenous dopamine) before being stored frozen. Each assay tube received 200 μl buffer, 200 μl [^{3}H]dopamine (0.7 nM final concentration; 34 Ci/mmole; New England Nuclear Corp.; stored at

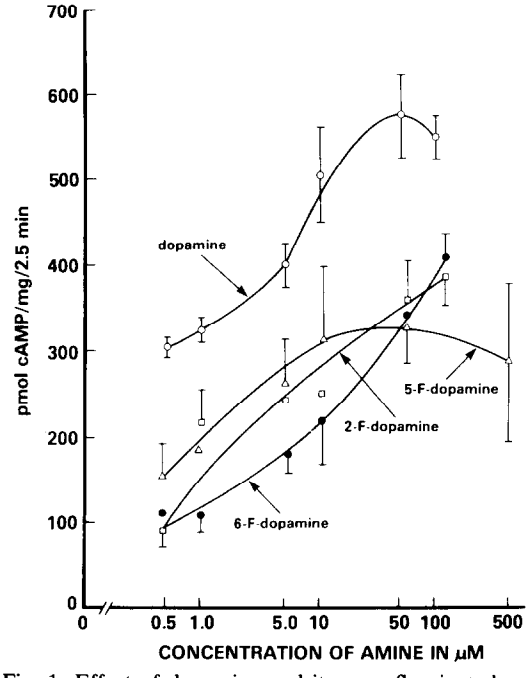

Fig. 1. Effect of dopamine and its monofluorinated analogues on adenylate cyclase activity in a homogenate of rat caudate nucleus. Data are means ± S.E.M. of at least three separate determinations run in triplicate. They represent adenylate cyclase activity above the control value of 180 ± 30 pmoles cAMP · mg^{-1} · 2.5 min^{-1}.

Table 1. Stimulation of adenylate cyclase in homogenates of rat striatum produced by fluoro-dopamines

Amine (10 μM)	pmoles Cyclic AMP produced·(mg protein)$^{-1}$·2.5 min^{-1}, above basal measured at pH 7.4			
	Control	With 10 μM haloperidol	With 10 μM phentolamine	With 10 μM propranolol
2-Fluoro-dopamine	236 ± 45	7 ± 2	156 ± 11	190 ± 12
5-Fluoro-dopamine	311 ± 120	19 ± 11	146 ± 28	276 ± 8
6-Fluoro-dopamine	220 ± 32	21 ± 3	137 ± 17	252 ± 22

Each value is the mean ± S.E.M. of three separate determinations run in triplicate. The basal value of cyclic AMP production was 216 ± 12 pmoles·(mg protein)$^{-1}$·2.5 min^{-1}.

500 nM in 0.1% ascorbate at −20°), and 200 μl tissue (0.5–0.6 mg protein per tube). After incubation at 20–21° for 30 min, 0.5 ml aliquots were vacuum-filtered through Whatman GF/B filters, followed by a wash with 10 ml buffer. The specific binding of [^{3}H]dopamine was defined as the binding to 200 nM apomorphine that was inhibited by 1 μM dopamine.

RESULTS

Effects of the fluoro-dopamines on dopamine-sensitive adenylate cyclase. As shown in Fig. 1, 2-, 5- and 6-fluoro-dopamine each stimulated dopamine-sensitive cyclase but were considerably less effective than dopamine. As detailed in Table 1, haloperidol (10 μM) almost completely antagonised the stimulating actions of these fluoro-dopamines at this D_1 receptor site. The alpha-adrenoceptor blocker, phentolamine, and the beta-adrenoceptor blocker, propranolol, were much less effective than haloperidol in inhibiting the stimulating activity of the fluoro-dopamines (Table 1).

The results in Table 2 give an example illustrating qualitatively that the fluoro-dopamines did not antagonize the action of dopamine on the adenylate cyclase but were additive to the stimulation elicited by dopamine.

Effects of the fluoro-dopamines on D_2 dopamine receptors. As mentioned in the introduction, the D_2 dopamine receptor is sensitive to micromolar concentrations of dopamine. The results in Table 3 are in accord with this general finding for the D_2 receptor site. Dopamine and the fluoro-dopamines all had IC_{50} values between 17,000 and 63,000 nM. The K_i values (dissociation constants for the inhibition of [^{3}H]spiperone binding) can be calculated from these IC_{50} values, using the Cheng–Prusoff relation [24]: $K_i = IC_{50}/(1 + C^*/K_D)$, where C^* was the concentration of [^{3}H]spiperone, and D_d was the dissociation constant for [^{3}H]spiperone. Since the value for C^*/K_D was generally 0.07 nM/0.25 nM, the K_i values were only about 25 per cent lower than the IC_{50} values.

Effects of the fluoro-dopamines on the D_3 receptor sites. In accordance with the definition of the D_3 dopamine-sensitive site (see Introduction), dopamine and the three fluoro-dopamines all revealed IC_{50} values of between 1 and 3 nM against the specific binding of [^{3}H]dopamine (Table 3). Since the value for C^*/K_D for these experiments was 0.7 nM/2 nM, the K_i values were all approximately 30 per cent lower than the IC_{50} values listed in Table 3.

DISCUSSION

The results indicate that the fluoro-dopamines were bound to and stimulated the D_1 receptor site (dopamine-sensitive adenylate cyclase) in the micromolar concentration range (Fig. 1), inhibited the binding of [^{3}H]spiperone to the D_2 receptor in the micromolar range (Table 3), and inhibited the binding of [^{3}H]dopamine to the D_3 receptor site in the nanomolar concentration range (Table 3). On the whole, these potencies for the fluoro-dopamines at the three different receptor sites were generally the same as those for dopamine.

The potencies of a variety of dopamine agonists for eliciting behavioural rotation (in unilateral nigra-lesioned rats), for emesis, for prolactin inhibition, and for stereotypy all correlate with the potencies of these compounds at the D_2 receptor and not at the D_1 or D_3 dopaminergic sites [12]. In fact,

Table 2. Additive effect of dopamine and 5-fluoro-dopamine on dopamine-sensitive adenylate cyclase

Amines	pmoles Cyclic AMP.(mg protein)$^{-1}$·2.5 min^{-1} above basal
Dopamine (DA) (5 μM)	693 ± 158
5-Fluoro-dopamine (10 μM)	311 ± 120
DA (5 μM) + 5-fluoro-DA (10 μM)	1450 ± 246

Each value is the mean ± S.E.M. of three separate determinations run in triplicate. The basal value of cyclic AMP production was 216 ± 12 pmoles·(mg protein)$^{-1}$·2.5 min^{-1}.

Table 3. Effects of fluoro-dopamines on D_2 and D_3 receptor sites

	D_2 site [^{3}H]Spiperone (0.07–0.1 nM) IC_{50} (nM)	D_3 site [^{3}H]Dopamine (0.7 nM) IC_{50} (nM)
Dopamine	17,000 ± 5,000	1.5 ± 0.4
2-Fluoro-dopamine	35,000 ± 5,000	1 ± 0.3
5-Fluoro-dopamine	63,000 ± 4,000	3 ± 0.6
6-Fluoro-dopamine	50,000 ± 6,000	1 ± 0.2

The IC_{50} value (mean ± S.E. for three to four experiments) is the concentration which inhibited by 50 percent the specific binding of each ^{3}H-ligand.

we have shown that the ability of 5-fluoro-dopamine to suppress prolactin release from pituitary cells in culture is the same as that of dopamine.* It is important, therefore, that the IC_{50} values of the fluoro-dopamines were approximately the same as the value for dopamine itself. As reviewed elsewhere in detail [12], the IC_{50} values for dopamine on the D_2 receptor (i.e. to inhibit the binding of [^{3}H]spiperone) are generally between 4,000 and 50,000 nM. The fluoro-dopamine IC_{50} values fell into this range, with the exception of 5-fluoro-dopamine which was 63,000 nM.

The greatest divergence between the potencies of dopamine and those of the fluoro-dopamines occurred at the D_1 receptor site (Fig. 1). It appears reasonable to think that this difference has something to do with the ionization state of the different dopamines. For example, at pH 7.4, dopamine ($pK_{OH} = 8.9$) is ionized to the extent of 3.2 per cent, while 5-fluoro-dopamine ($pK_{OH} = 8.0$) is ionized by 20.1 per cent.

The biological role of the D_3 receptor site is unclear. Indirect evidence suggests that this receptor site may have some pre-synaptic function in the dopamine pathway [15, 25]. If so, the fluoro-dopamines would be expected to exert similar actions on that pre-synaptic system, since the IC_{50} values were practically identical to that for dopamine (Table 3). It has already been noted [14, 15] that the D_3 receptor site herein labelled by [^{3}H]dopamine is very different from the receptor site being labelled by [^{3}H]apomorphine when one employs the conditions of Creese *et al.* [26]. The receptor site labelled by the method of Creese *et al.* has characteristics (i.e. IC_{50} values) resembling the D_2 receptor, namely, the IC_{50} value for dopamine is of the order of 0.3 μM [15]. Thus, the recent values of the fluoro-dopamines for inhibiting the binding of [^{3}H]apomorphine (IC_{50} values of between 0.1 and 0.4 μM) presumably apply to either the D_2 receptor or a mixture of the D_2 and D_3 receptor sites [27].

In conclusion, the approximately similar IC_{50} values for dopamine and the fluoro-dopamines at the D_2 receptor further indicate that ^{18}F-labelled compounds may be valid markers to study dopamine function by non-invasive means.

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