Pharmacological and Biochemical Evidence for the Existence of Two Categories of Dopamine Receptor

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ABSTRACT: Evidence supporting the validity of the 'two dopamine receptor' hypothesis is presented. The availability of the 'first generation' of selective agonists and antagonists of the D-1 and the D-2 dopamine receptors provides pharmacological support for the hypothesis. The demonstration that stimulation of the D-2 receptor either inhibits or has no effect upon adenylate cyclase activity while stimulation of the D-1 receptor enhances cAMP formation provides biochemical support for the hypothesis. Finally, binding assays demonstrating two affinity states for the D-1 and the D-2 receptors are briefly discussed.

RÉSUMÉ: Nous présentons nos évidences en faveur de l'hypothèse des "deux récepteurs dopaminergiques". L'appui pharmacologique provient des agonistes et antagonistes spécifiques aux récepteurs D_1 et D_2 de la première génération. Ainsi nous montrons que la stimulation du récepteur D_1 augmente la formation d'AMP cyclique alors que celle du récepteur D_2 inhibe ou n'a aucun effet sur l'activité de l'adenylate cyclase. Enfin nous discuttons brièvement des tests de liaison qui démontrent deux états d'affinité pour chacunes des formes de récepteurs.

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In 1979, the existence of two categories of dopamine receptor was hypothesized (Kebabian and Calne, 1979). This 'two dopamine receptor' hypothesis was formulated in an attempt to account for the unanticipated observation that two ergots, lisuride and lergotrile, mimicking the action of dopamine upon the anterior pituitary gland blocked the stimulatory effect of dopamine upon adenylate cyclase activity of homogenates of the caudate nucleus of the rat brain (Kebabian et al., 1977; Pieri et al., 1978). Table 1 presents a summary of the salient features of this hypothesis as it was put forward in 1979. In addition to the aforementioned ergots, only a limited number of drugs discriminated between the two categories of dopamine receptor. An additional difference between the two receptors was the biochemical consequences of receptor stimulation. Stimulation of the D-1 receptor enhanced the formation of cAMP; in the bovine parathyroid, a dopamine-enhanced formation of cAMP appeared to be involved in the physiological response to this amine (Brown et al., 1977, 1980). In contrast, stimulation of the D-2 receptor did not appear to enhance cAMP production; in the anterior pituitary gland, a dopamine-enhanced formation of cAMP did not appear to be involved in the physiological response to dopamine. This latter situation lead to the designation "cyclase linkage: No" for the D-2 receptor.

Since this hypothesis was put forward, several other investigators proposed the existence of as few as 1 or as many as 4 dopamine receptors (Laduron, 1983; Seeman, 1980; Sokoloff et al., 1980). The existence of these apparently conflicting classification schemata prompted testing of the validity of the two dopamine receptor hypothesis in three types of experiments. First, selective agonists and antagonists of the D-1 and the D-2 dopamine receptor have been identified in pharmacological experiments. Second, the consequences of stimulating the D-2 receptor have been determined in biochemical and physiological experiments. Third, binding assays have been developed which

Table 1: Elements of the "two dopamine receptor" hypothesis as initially presented in 1979 — modified from Kebabian and Calne (1979)

Name*	D-1	D-2
Cyclase linkage	Yes	No
Location of prototype receptor	Bovine parathyroid	Mammotroph of anterior pituitary
Dopamine	Agonist (μmolar potency)	Agonist (nmolar potency)
Apomorphine	Partial agonist or antagonist	Agonist (nmolar potency)
Dopaminergic ergots	Potent antagonist nmolar potency) Weak agonist (μmolar potency)	Agonist (nmolar potency)
Selective antagonist	None known as yet	Metoclopramide sulpiride
Radiolabelled ligand	*cis*-flupenthixol†	Dihydroergocryptine

*Previously, the two categories of dopamine receptors were designated as 'α-dopaminergic' and 'ß-dopaminergic'. This has led to confusion with the α and ß adrenoreceptors. The new designations should prevent further confusion.
†Radiolabelled *cis*-flupenthixol can be used as a ligand specific for the dopamine receptor linked to adenylyl cyclase in the rat striatum. Its affinity for the dopamine receptor in the anterior pituitary has not been measured.

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are capable of identifying either the D-1 or the D-2 dopamine receptor. Data supporting the validity of the 'two dopamine receptor' hypothesis has been obtained from each of these endeavors.

Selective D-1 and D-2 Agonists and Antagonists

The development of stereoselective agonists and antagonists discriminating between the D-1 and the D-2 dopamine receptors has provided pharmacological verification of the two dopamine receptor hypothesis. Several drugs selective for the D-1 receptor have come from the benzazepines (Figures 1 and 2). The first selective D-1 agonist, SKF 38393, was developed by Smith Kline and French Laboratories (Setler et al., 1978). The two optical isomers of SKF 38393 have been synthesized and their pharmacological activity evaluated. The D-1 agonist activity resides in the (R)-isomer; the (S)-isomer is significantly less potent (Kaiser, 1983). Recently, another selective D-1 agonist, designated as SKF 82526, has been synthesized as a potential antihypertensive agent (Hahn et al., 1982). Furthermore SCH 23390, the 7-chloro, N-methyl analogue of SKF 38393, has been identified as a selective antagonist of the D-1 receptor (Iorio et al., 1983; Hyttel, 1983). In addition, SKF 83509, the 7-chloro analogue of SKF 38393, also selectively blocks the D-1 receptor (Itoh, Beaulieu and Kebabian, in preparation). The D-1 antagonist activity of both molecules is a consequence of the 7-chloro substituent which diminishes the efficacy of the compound as an agonist and increases the affinity of the molecule towards the D-1 receptor (Itoh, Beaulieu and Kebabian, in preparation).

Several compounds have been identified as selective agonists upon the D-2 receptor. Both LY 141865 as well as RU 24926 mimic the effect of dopamine upon the D-2 receptor in the pituitary gland. However, neither of these compounds mimic the ability of dopamine to stimulate adenylate cyclase; therefore, these compounds represent the selective D-2 agonists (Euvrard et al., 1980; Tsuruta et al., 1981). Recently, the structure of the active isomer of LY 141865 has been identified (Titus et al., 1983). There are many antagonists which selectively block the D-2 receptor. Both domperidone and YM-09151-2 are significantly more potent antagonists of the D-2 receptor than is (-)-sulpiride (Denef and Follenbouckt, 1978; Grewe et al., 1982).

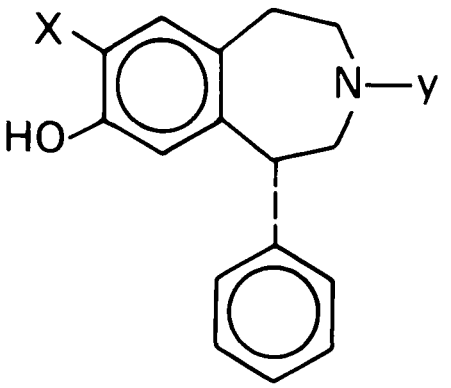
The compounds identified in figure 1 are the 'first generation' of selective dopaminergic drugs. Their existence provides support for the validity of the 'two dopamine receptor' hypothesis. In addition, these compounds permit the identification of the structural features responsible for their selective properties. It seems reasonable to anticipate that additional agents possessing a higher degree of selectivity will be developed in the future.

Consequences of stimulating the D-2 receptor

The pituitary gland has provided a biochemical model of the D-2 receptor. Using tissue of the intermediate lobe of the pituitary gland, stimulation of the D-2 receptor has been shown to diminish adenylate cyclase activity; this dopaminergic inhibition of

	D-1	D-2
Adenylate Cyclase	Enhances	Inhibits or No effect
Example	Bovine Parathyroid Fish Retina Neostriatum	Pituitary gland Autoreceptor Cholinergic Interneurons
Agonist	SKF 38393	LY 141865 RU 24926
Antagonist	SCH 23390 SKF 83509	YM 90151-2 Domperidone (−) Sulpiride

Figure 1 — Elements of the "two dopamine receptor" hypothesis as of 1983. The most salient feature is the identification of the "first generation" of selective agonists and antagonists for the two categories of dopamine receptor.



X	y	
OH	H	SKF 38393
OH	CH_3	SKF 75670
Cl	H	SKF 83509
Cl	CH_3	SCH 23390

Figure 2 — Structure of several selective D-1 agonists and antagonists. Both SKF 38393 and SKF 75670 are agonists upon the D-1 receptor. In contrast, both the 7-chloro-substituted benzazepines are antagonists of the D-1 receptor.

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adenylate cyclase requires the presence of GTP in the enzyme assay system (Cote et al., 1982). The inhibitory effect of dopaminergic agonists is especially pronounced when the enzyme activity is enhanced with a stimulatory agent such as isoproterenol, cholera toxin, corticotropin releasing factor or forskolin (Cote et al., 1982; Meuniere and Labrie, 1982; Meuniere et al., 1982; Miyazaki et al., 1984). In the anterior pituitary gland, a similar dopaminergic inhibition vasoactive intestinal peptide-stimulated adenylate cyclase activity been demonstrated (Onali et al., 1981).

An example of D-2 receptor inhibiting adenylate cyclase activity has been identified in the central nervous system. In the neostriatum of the rat brain, D-2 agonists inhibit the efflux (and by inference, the synthesis of cAMP from intact tissue (Stoof and Kebabian, 1981, 1982). However, the anatomical complexities of the brain limits the precision with which the biochemical consequences of stimulating the receptor can be identified.

Three physiological consequences of stimulating the D-2 receptor in the intermediate lobe of the rat pituitary gland have been identified. Stimulation of this receptor decreases: the synthesis of cyclic AMP (Munemura et al., 1980); the spontaneous regenerative electrical activity across the plasma membrane (Douglas and Taraskevich, 1978, 1980, 1982); and the release of alpha-melanocyte stimulating (αMSH)-like peptides (Munemura et al., 1980; Meuniere and Labrie, 1982). The inhibition of cAMP production can be attributed to the inhibition of adenylate cyclase activity. However, the relative importance of diminished cAMP synthesis and the inhibition of electrical activity in producing the inhibition of αMSH-like peptides remains to be determined. On the one hand, Douglas and Taraskevich (1980, 1982) provide evidence suggesting that the dopaminergic inhibition of electrical activity could diminish the amount of calcium ions entering the melanotroph; this diminished entry of calcium would inhibit the release of hormone from the cells. On the other hand, agents either promoting the synthesis or inhibiting the breakdown of cAMP enhance the calcium-dependent release of hormones from the intermediate lobe (Munemura et al., 1980). However, since removal of calcium from the extracellular environment blocks the release of hormone but does not inhibit the synthesis of cAMP, it may be concluded the cAMP does not trigger the release of hormone (Tsuruta et al., 1982). Reuter (1983) has suggested that in the heart cAMP may promote the entry of calcium into the myocardium; it remains to be determined if this mechanism exists in the pituitary gland.

The data obtained from the pituitary gland and brain indicated that stimulation of the D-2 receptor diminishes the synthesis of cAMP. This evidence is in accord with the view that certain "physiological responses to dopamine seem not to involve either the stimulation of an adenylyl cyclase or the accumulation of intracellular cyclic AMP" (Kebabian and Calne, 1979). However, it is also clear that the designation "cyclase linkage: No" for the D-2 receptor (Table 1) is no longer appropriate. However, in the neostriatum, a D-2 receptor occurs upon the acetylcholine-containing neurons as well as upon the terminals of the dopamine-containing nigro-neostriatal neurons. Stimulation of these receptors inhibits the depolarization-induced release of either acetycholine or dopamine. There is no evidence to suggest that cAMP participates in either of these physiological responses (Stoof, 1983).

Binding assays identifying the D-1 and D-2 dopamine receptors

Numerous dopaminergic drugs, radiolabeled to high specific activity, have been developed for identifying dopamine receptors in binding assays. In order to account for the discrepancies obtained in binding assays using different ligands, the existence of as few as one or as many as four different dopamine receptors has been proposed (Laduron, 1983; Seeman, 1980; Sokoloff, 1980). However, an alternative interpretation of these differences has recently been formulated by Creese. Sibley et al. (1982) propose that the D-2 receptor in the pituitary gland exists in two interconvertible states displaying different properties in binding assays. Furthermore, Leff and Creese (1983) report that the D-1 receptor may also exist in two states. According to this interpretation, the four receptors seen in binding assays represent the two states of the D-1 and the D-2 receptors. In accord with this possibility, Hyttel (1983) has recently claimed to have identified the D-1 receptor in a binding assay.

Conclusion

The classification of dopamine receptors remains an area of controversy among different investigators. The 'two dopamine receptor' hypothesis has been subjected to extensive testing in the 5 years since its formulation. The data obtained in many laboratories can be most easily interpreted within the guidelines provided by this hypothesis.

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