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Inhibition of Prolactin Release from Anterior Pituitary Lactotrophs in Culture by Sulfur-Containing Analogs of Dopamine (42711)

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Abstract. The effects of permanently charged and uncharged analogs of dopamine were examined for their ability to inhibit basal prolactin release from primary cultures of rat pituitary lactotrophs. The charged quaternary trimethyldopamine and the charged dimethylsulfonium analogs were active (IC_{50}'s were 4.3 and 31 μM, respectively) while the permanently uncharged monoethylsulfide was devoid of significant activity. Dopamine and dimethyldopamine, which are able to exist in both charged and uncharged forms, are more potent (IC_{50}'s were 36 and 44 nM, respectively) but all compounds were capable of approaching the same maximum degree of prolactin release inhibition. Haloperidol, a dopamine receptor antagonist, was able to block the actions of each of the agonists. The data suggest that (a) dopamine agonists do not have to be in the uncharged form in order to activate the dopamine receptor that regulates prolactin release, (b) the uncharged monomethylsulfide analog of dopamine is incapable of activating the dopamine receptor, and (c) the nitrogen on the side chain of dopamine can be replaced by another atom and still retain prolactin release inhibiting activity.

Dopamine and dopaminergic agonists contain an amine moiety which exists in physiological solution in both charged and uncharged forms. At present, it is not clear which of these molecular species interact with the recognition site of the dopamine receptors. It has been previously suggested that the uncharged forms of dopamine and dopamine receptor agonists (and antagonists) interact with the dopamine receptor (1). However, this assertion was based mainly on studies of the effects of agonist and antagonist drugs which were primary, secondary, and tertiary amines. Since these amines are present in physiological solution in both charged and uncharged forms, the species which interacts with the receptor cannot be determined with certainty.

Anderson *et al.* (2) have synthesized analogs of dopamine in which the amine group is replaced by either a dimethylsulfonium group, which carries a permanent positive electrostatic charge, or a methylsulfide group, which is permanently uncharged. The abilities of these two compounds to activate D-2 dopamine receptors were evaluated by determining whether they were able to inhibit the potassium-evoked release of [^{3}H]-acetylcholine from striatal slices *in vitro*. In these studies, the permanently charged dimethylsulfonium analog displayed dopamine agonist activity, while the uncharged methylsulfide analog was inactive (3). In order to measure "direct" receptor activity in this model system it was necessary to pretreat the animals with reserpine (to deplete endogenous catecholamine stores) and α-methyl-*p*-tyrosine (to inhibit dopamine synthesis). In addition, it was necessary to add α-methyl-*p*-tyrosine to the incubation medium. It is possible that these drug pretreatments and drug additions affected the response of the striatal tissue to the dopamine analogs in ways which are not fully appreciated.

Lactotroph cells of the anterior pituitary gland contain dopamine D-2 receptors which may participate in the regulation of prolactin release from intracellular stores (4). Since these cells are not innervated by dopamine-containing neurons, they can be used as a model system to study D-2 receptor activity without the complications associated with the presence of potential prejunctional sites of action and without the need for drug pretreatments to eliminate endogenous sources of dopamine. Therefore we have investigated the effects of the sulfur-containing analogs of dopamine on the basal release of prolactin from anterior pituitary lactotrophs in monolayer culture in order to evaluate

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their direct D-2 agonist activity. For comparison purposes, the effects of dopamine, dimethyldopamine, the quaternary trimethyldopamine, and apomorphine were also examined. The selectivities of the drugs' dopaminergic actions were also evaluated in the presence of haloperidol, a D-2 receptor antagonist.

Methods. *Chemicals.* (2-(3,4-Dihydroxyphenyl)ethyl)dimethylsulfonium iodide, (2-(3,4-dihydroxyphenyl-ethyl)methylsulfide, *N,N*-dimethyldopamine HBr, and *N,N,N*-trimethyldopamine iodide were synthesized in the laboratory of D. D. Miller, Division of Medicinal Chemistry, Ohio State University.[1] The structures of these compounds are contrasted with that of dopamine in Fig. 1. The dimethylsulfonium compound is a catechol with a permanently charged side chain while the monomethylsulfide has a permanently uncharged side chain. Stock solutions of the uncharged compound, which was poorly soluble in water, were prepared using a multisolvent vehicle containing 95% ethanol, dimethylsulfoxide, and propylene glycol 400 in a ratio of 1:1:3. The stock solution was subsequently diluted with water or culture medium. Apomorphine HCl and dopamine HCl were obtained from Sigma Chemical Co. (St. Louis, MO); haloperidol was from McNeil Pharmaceutical (Spring House, PA).

Anterior pituitary cell culture. Pituitary glands were obtained from 200- to 300-g female Sprague–Dawley derived rats (Harlan Industries, Indianapolis, IN) maintained in a climate-controlled, AAALAC certified vivarium. Isolation and culturing techniques were those described by Kim and Burkman (5).

Experimental protocol. Well-established, preconfluent, primary cell cultures underwent a medium exchange (Earle's minimum essential medium containing 10% fetal calf serum and supplemented with 0.1 m*M* nonessential amino acids, 2 m*M* L-glutamine, 1 m*M* sodium pyruvate, 26 m*M* sodium bicarbonate, and 40 μg/ml gentamicin) and at the end of a 5-hr pretreatment period, a 50-μl sample of culture medium was removed. Test drugs were added in 2- to 10-μl volumes and at the end of a 5-hr treatment period, another aliquot was removed. Pre- and posttreatment samples were diluted with 1% buffered bovine serum albumin and stored in a frozen state (−25°C). The samples were subsequently assayed for their contents of prolactin. Pretreatment concentrations of prolactin were subtracted from posttreatment determinations in order to estimate the amount of hormone released during the treatment period. The amounts of prolactin released during the treatment period were compared with those derived from parallel cultures exposed to the control vehicle. Treatment response was finally expressed as percentage inhibition of control prolactin level. Cells were exposed to varying concentrations of the test compounds so that log dose–response curves could be constructed using a nonlinear curve-fitting program (6). The construction of each dose–response curve required a minimum of 18–36 culture wells.

Assay of prolactin. Rat prolactin concentrations in the culture media were determined by a double antibody radioimmunoassay using the protocols of the NIH National Hormone and Pituitary Program. Concentrations of prolactin were interpolated from a standard curve which was constructed using NAIDDK rat prolactin RP-3.

Results and Discussion. *Effect of apomorphine, dopamine, dimethyldopamine, and the quaternary ammonium, dimethylsulfonium, and monomethylsulfide analogs of dopamine on prolactin release.* After incubation of the cultured lactotrophs with the drugs listed above and control vehicles for 5 hr, aliquots of the medium were removed and assayed for prolactin by RIA. Table I summarizes the median inhibitory concentrations (IC_{50}'s) of each of the compounds examined, the maximum degree of inhibition, and the magnitude of their associated experimental errors interpolated from the log dose–response curves portrayed in Fig. 2. As previously demonstrated by others, both apomorphine and dopamine inhibited prolactin release. Our IC_{50} of 17 n*M* for apomorphine is somewhat higher than the

[1] IR and NMR spectroscopic and elemental analyses data for the synthesized compounds deviate from the theoretical values by no more than ±0.4%.

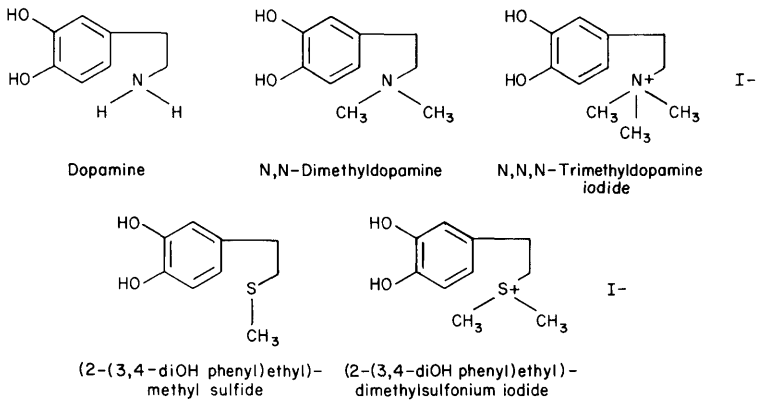

FIG. 1. Dopamine and dopamine analogs.

2.9–3.0 n*M* estimates of Foord *et al.* (7) and Caron *et al.* (8). The IC_{50} of dopamine (36 n*M*), on the other hand, compared favorably with those reported by these investigators (30–35 n*M*). Dimethyldopamine inhibited prolactin release with an IC_{50} of 44 n*M*, a value not substantially different from that of dopamine. The permanently charged quaternary ammonium and dimethylsulfonium analogs were clearly active generating full dose–response curves although they were only about 0.01 and 0.001 times the potency of dopamine, respectively (Table I). In contrast, the permanently uncharged monomethylsulfide analog did not significantly alter prolactin release at concentrations up to 100 μM.

Effect of haloperidol on the inhibition of prolactin release induced by dopamine and the permanently charged analogs of dopamine. In order to determine whether the inhibition of prolactin release was due to the activation of dopamine receptors, the effects of the agonists were reexamined in the presence of 100 n*M* haloperidol, a dopamine receptor antagonist. This dose of haloperidol had been found to produce a 95% reduction in dopamine's action on the prolactin system (9). Table II illustrates that haloperidol dramatically reduces the actions of dopamine and both the nitrogen- and the sulfur-bearing analogs.

TABLE I. MEDIAN INHIBITORY CONCENTRATION (AND THEIR STANDARD ERROR LIMITS) AND MAXIMUM DEGREES OF INHIBITION OF PROLACTIN RELEASE ASSOCIATED WITH DOPAMINE RECEPTOR AGONISTS AND ANALOGS

Compound	IC_{50} (SE limits) (n*M*)	Inhibition maximum (%)
Dopamine	36 (23–56)	96 ± 12[e]
Apomorphine	17 (8–36)	110 ± 23
Dimethyl DA[a]	44 (34–56)	78 ± 7
Trimethyl DA[b]	4,300 (2,630–7,240)	85 ± 21
Monomethyl S[c]	—	0
Dimethyl S[d]	30,700 (16,980–56,230)	101 ± 37

[a] *N,N*-Dimethyldopamine.
[b] *N,N,N*-Trimethyldopamine iodide.
[c] (2-(3,4-Dihydroxyphenyl)ethyl)methylsulfide.
[d] (2(3,4-Dihydroxyphenyl)ethyl)dimethylsulfonium iodide.
[e] SE.

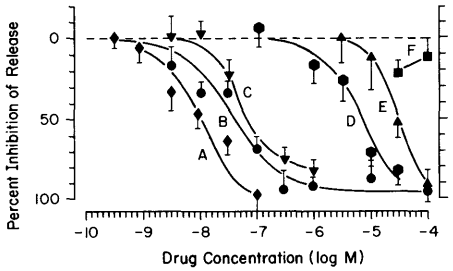

FIG. 2. Dose-dependent inhibition of pituitary prolactin release by dopamine, dopamine analogs, and apomorphine. Each point is the mean (±SE) of three to six replicate determinations. (A) Apomorphine; (B) dopamine; (C) *N,N*-dimethyldopamine; (D) *N,N,N*-trimethyldopamine; (E) dimethylsulfonium analog; (F) monomethylsulfide analog.

TABLE II. HALOPERIDOL ANTAGONISM OF THE INHIBITION OF PROLACTIN RELEASE BY DOPAMINE AND ITS NITROGEN AND SULFUR ANALOGS

Compound	Percentage inhibition ± SE	
	Without haloperidol	With haloperidol[a]
Dopamine (1 μM)	92 ± 10 (12)[e]	11 ± 18 (12)
Dimethyl DA (0.1 μM)[b]	69 ± 7 (6)	18 ± 14 (6)
Trimethyl DA (10 μM)[c]	75 ± 9 (6)	8 ± 12 (6)
Dimethyl S (100 μM)[d]	90 ± 10 (6)	18 ± 15 (6)

[a] One-hundred nanomolar co-incubated with the agonist during the treatment period.
[b] *N,N*-Dimethyldopamine.
[c] *N,N,N*-Trimethyldopamine iodide.
[d] (2-(3,4-Dihydroxyphenyl)ethyl)dimethylsulfonium iodide.
[e] Number of replicate determinations (culture wells).

The results indicate that catechols with permanently charged side chains can activate D-2 dopaminergic receptors that influence the release of pituitary prolactin, while catechols with a permanently uncharged side chain cannot. Thus, both the dimethylsulfonium and the quaternary ammonium analogs can inhibit prolactin release. The maximum degree of release inhibition produced by these compounds was not significantly different from that produced by dopamine and apomorphine. The inhibition of prolactin release was antagonized by coincubation with the competitive dopamine receptor inhibitor haloperidol. These observations clearly suggest that the side chain of a dopamine agonist does not have to be in an uncharged state in order to bind to and activate the dopamine receptor that regulates prolactin release. They also indicate that the nitrogen on the side chain of dopamine can be replaced with other atoms and still retain D-2 dopaminergic activity.

In contrast, the uncharged monomethylsulfide analog failed to activate dopamine receptors on lactotroph cells. This observation is in agreement with recent studies showing that compounds having uncharged side chains were unable to directly activate the D-2 receptors that inhibited potassium-induced acetylcholine release in the striatum (10, 11). A positive charged side chain, therefore, appears to be essential for the activation of dopamine receptors and although dopamine can exist in physiological solution as both charged and uncharged species, it is the charged amine form that actually interacts with an anionic site on the receptor and that results in activation. Indeed, the lack of a charge would appear to render the compound incapable of activating the dopamine receptor.

It was observed that the charged quaternary ammonium and dimethylsulfonium compounds were less potent than dopamine, dimethyldopamine, and apomorphine. This suggests that the ability of the latter compounds to exist in both charged and neutral states may enhance their affinity for or facilitate their transport to the receptor. For such compounds, the uncharged species may facilitate interaction with the lipid membrane and thereby enhance access of the molecule to the receptor. The permanently charged molecule, therefore, may be seen as lacking a physicochemical attribute that is necessary for optimal potency. Nevertheless, the permanently charged compounds may have characteristics that increase their specificity of action. For example, since the dimethylsulfonium and quaternary ammonium compounds are permanently charged, they are unlikely to possess sufficient lipid solubility to effectively cross the blood brain barrier. When administered systemically, these substances would probably not be of value in influencing disorders of the brain associated with dopamine deficiency. However, since the anterior pituitary gland is outside the blood brain barrier, these compounds may be useful in inhibiting prolactin release under conditions that are characterized by prolactin hypersecretion (e.g., pituitary adenoma, galactorrhea) without provoking the central adverse effects commonly associated with dopamine agonists.

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