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EFFECT OF PERMANENTLY CHARGED AND UNCHARGED DOPAMINERGIC AGONISTS ON THE POTASSIUM-INDUCED RELEASE OF [^{3}H]ACETYLCHOLINE FROM STRIATAL SLICES

BRIAN SABOL, ROBERT BOLDRY, TAHIRA FAROOQUI, YU AN CHANG, DUANE MILLER and NORMAN URETSKY*

Divisions of Pharmacology and Medicinal Chemistry, College of Pharmacy, The Ohio State University, Columbus, OH 43210, U.S.A.

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Abstract—The effects of chemical analogs of dopamine, which are permanently charged or which lack a net positive charge, on the potassium-evoked release of [^{3}H]acetylcholine from mouse striatal slices were studied in order to determine whether a positive charge on the dopamine agonist molecule is required to activate dopaminergic receptors. The striatal slices were first preincubated with [^{3}H]choline, transferred to a superfusion chamber, and then superfused in physiological medium. [^{3}H]Acetylcholine release was evoked by exposure of the slices to a high potassium medium and potential dopamine agonist drugs were added to the medium 10 min before superfusing with high potassium. A permanently charged quaternary ammonium analog and dimethylselenonium analog of dopamine inhibited the potassium-evoked release of [^{3}H]acetylcholine, and this inhibition was antagonized by sulpiride, a dopamine receptor antagonist. However, this inhibition was not antagonized by reserpine and α-methyl-*p*-tyrosine, which was shown to completely antagonize the inhibitory effect of amphetamine, an indirectly acting amine. This suggests that the charged dopamine analogs are acting directly on dopaminergic receptors. In contrast to the permanently charged dopamine analogs, analogs of dopamine with no net positive charge produced no inhibition of the potassium-evoked [^{3}H]acetylcholine release. These *in vitro* observations are in agreement with a behavioral model in which a permanently uncharged monomethylsulfide analog of dopamine was ineffective in eliciting circling behavior after its unilateral injection into the striatum of rats in which dopamine neurons were previously lesioned by the injection of 6-hydroxydopamine into the medial forebrain bundle. In contrast, under these same conditions, the intrastriatal injection of the charged quaternary ammonium or dimethylsulfonium analog of dopamine elicited intense contralateral circling. These results suggest that the charged form of a dopamine agonist molecule is required to bind to and activate the dopamine receptor regulating [^{3}H]acetylcholine release and circling behavior.

Dopaminergic agonists have been studied for potential use in a variety of pathological conditions [1–5]. As a result, the molecular requirements for the activation of dopamine receptors have been a subject of considerable study [4–8]. We have been interested in whether the side chain nitrogen of dopamine is necessary for dopamine agonist activity. Therefore, we have synthesized and studied the effects of a chemical analog of dopamine in which the amine group on the α-carbon of the dopamine molecule is replaced by a dimethylsulfonium group [9]. It was found that this compound was able to inhibit the depolarization-evoked release of [^{3}H]acetylcholine from striatal slices by both directly activating dopaminergic receptors as well as by increasing the synaptic concentration of dopamine [10]. Since the dimethylsulfonium analog of dopamine contains a permanent positive charge, the agonist action of this compound on neurons regulating [^{3}H]acetylcholine release suggests that a charged molecule is capable of activating dopaminergic receptors. Dopamine and dopamine agonists are amines which exist in physiological solution (pH 7.4) in both charged and uncharged forms. Therefore, the observed agonist action of a permanently charged molecule would suggest that the charged form of dopamine and dopamine agonist molecules may bind to and activate the dopaminergic receptor on striatal cholinergic neurons.

At the present time, this hypothesis has only been tested using the permanently charged dimethylsulfonium analogs of dopamine. It is, therefore, possible that the dopamine agonist activity of these compounds may be a unique property of the dimethylsulfonium group. In addition, the extent of direct dopamine receptor activation by the dimethylsulfonium analog is unclear, since its agonist action appears to be, in part, mediated by endogenous dopamine [10]. Finally, although there is evidence that a permanently charged molecule can directly activate dopaminergic receptors, there is no information on whether a charge on the molecule is *required* for this effect. In the present study, we show that permanently charged analogs of dopamine (Fig. 1), other than the dimethylsulfonium analog, possess direct dopamine agonist activity, and permanently uncharged analogs of dopamine are inactive as dopaminergic agonists.

* Send correspondence to: Dr. Norman Uretsky, The Ohio State University, College of Pharmacy, 500 West 12th Ave., Columbus, OH 43210.

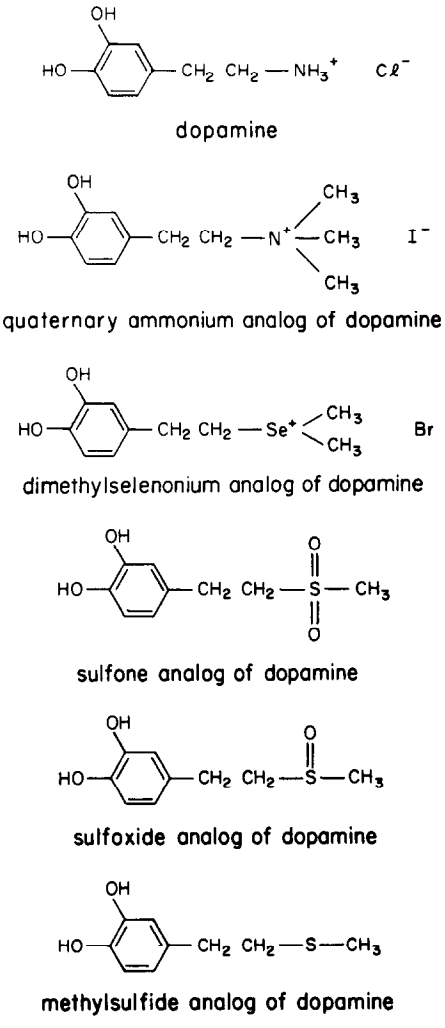

Fig. 1. Chemical structures of the analogs of dopamine that were studied.

MATERIALS AND METHODS

Materials. The sulfonium, sulfone, sulfoxide, sulfide and quaternary ammonium analogs of dopamine were prepared in our laboratory. The structural formulas of these compounds are shown in Fig. 1. The dimethylselenonium analog was supplied by Dr. S. A. Sadek, Professor of Medicinal Chemistry, University of Oklahoma. [*Methyl*-^{3}H]Choline chloride (15 Ci/mmol) was purchased from Amersham International Ltd. (Amersham, U.K.) The sulpiride and dopamine were purchased from the Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Preparation of striatal slices. Male Swiss–Webster mice (Harlan Swiss–Webster, Indianapolis, IN) were used for all experiments. After decapitation, the brains were removed, and the striatal tissue that was rostral to the anterior commissures was dissected [11]. The tissue was cut into 0.5 mm × 0.5 mm sections using a McIlwain tissue chopper (Brinkmann Instruments Inc., Westbury, NY) and dispersed into a Krebs–Ringer bicarbonate medium. The medium contained (mM): NaCl, 118; KCl, 4.8; $CaCl_2$, 1.3; $MgSO_4$, 1.2; $NaHCO_3$, 25; KH_2PO_4, 1.2; ascorbic acid, 0.6; disodium EDTA, 0.03; and glucose, 11. It was bubbled with a 95% O_2–5% CO_2 mixture and adjusted to a pH of 7.2 with NaOH. The slices were incubated for 20 min in this medium with [^{3}H]choline (0.1 μM) at 37°. This low concentration of [^{3}H]choline favors the selective uptake of choline into cholinergic neurons by a high-affinity uptake system [12]. After rinsing, the slices were transferred to a superfusion apparatus [13] and superfused with medium at 37° at a constant rate of 0.5 ml/min. Fractions were collected every 5 min, starting at 30 min after the onset of the superfusion. [^{3}H]Acetylcholine release was stimulated by superfusion with medium containing 13.7 mM K^+ for 10 min (2–5 min collection periods) at 50 min after the onset of the superfusion. The potential dopamine agonist drugs were added to the superfusion medium 10 min before the addition of the high K^+ medium (at 40 min; see Fig. 2). Sulpiride (2 μM), when present, was added at the start of the superfusion. In some studies, striatal slices were prepared from mice pretreated with reserpine (5 mg/kg, i.p.) and α-methyl-*p*-tyrosine (200 mg/kg, i.p.) at 20 and 1.5 hr, respectively, before the animals were killed. These slices were then superfused in medium containing α-methyl-*p*-tyrosine (0.25 mM). At the end of the superfusion, the radioactivity remaining in the tissue was extracted by homogenizing the tissue in 0.4 N perchloric acid. The radioactivity in the superfusate samples and tissue extracts was determined by liquid scintillation counting.

The outflow of tritium into the superfusion medium during each 5-min interval is expressed as a fraction of the total tritium content of the tissue at the beginning of the interval (fractional release).

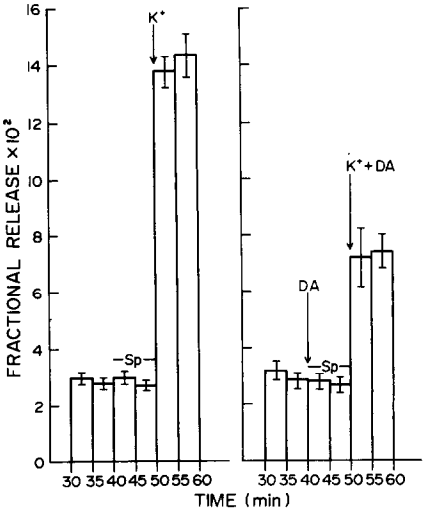

Fig. 2. Effect of dopamine on the K^+-induced release of [^{3}H]acetylcholine from striatal slices. Sp represents the mean spontaneous release of tritium during the two 5-min intervals preceding the addition of high potassium medium. Dopamine (10 μM) was added to the medium 10 min before the addition of the high K^+ medium. Each value is the mean ± SEM of five determinations.

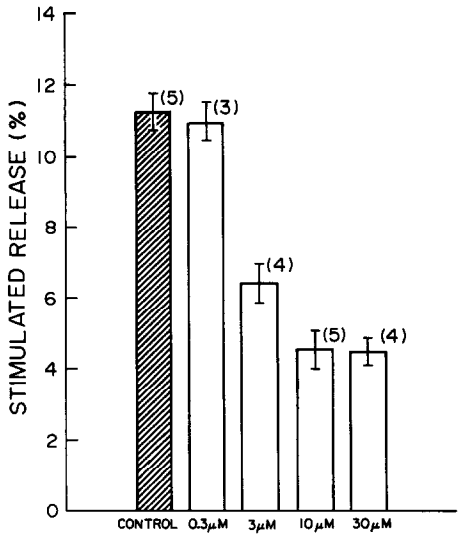

Fig. 3. Effect of different concentrations of dopamine on the K^+-induced release of [^{3}H]acetylcholine from striatal slices. Each value is the mean ± SEM of the fractional release evoked by the high K^+-medium above the spontaneous release.

This was calculated by correcting the tissue content of each fraction for the radioactivity lost to the medium. The K^+-evoked release is the average fractional release obtained following the addition of the high K^+ medium (2–5 min intervals) above the average spontaneous release (see Fig. 2). The latter is the average fractional release of tritium for the two 5-min intervals prior to the addition of the high K^+ medium. The effect of dopaminergic agonists on K^+-evoked release was evaluated by comparing the K^+-stimulated release in the presence of the dopaminergic agonist to that of control slices in which a dopamine agonist was not added to the medium (Fig. 2).

The tritium released by the high K^+ medium was not chemically characterized in these experiments. However, several previous studies have demonstrated that radioactive acetylcholine formed from radiolabeled choline can be released from brain slices by K^+-depolarization [14–20]. In these studies, physostigmine was added to the medium to inhibit the metabolism of acetylcholine. However, the inhibition of acetylcholine metabolism can cause high extracellular levels of acetylcholine which have been shown to inhibit the depolarization-induced release of acetylcholine by the process of feedback inhibition [19, 21]. In the present study, physostigmine was omitted from the medium. Under the conditions of this study, the K^+-stimulated release of tritium was completely dependent on the presence of calcium ions in the superfusion medium (data not shown).

Circling behavior in 6-hydroxydopamine-lesioned rats. The effects of potential dopaminergic agonists on circling behavior were determined by the direct injection of compounds into the caudate nucleus on the same side of the brain as a unilateral 6-hydroxydopamine-induced lesion of the medial forebrain bundle [22]. Rats (180–200 g, Harlan Sprague–Dawley, Indianapolis, IN) were anesthetized with chloral hydrate (400 mg/kg i.p.) and placed into the stereotaxic frame, and holes were drilled into the skull. 6-Hydroxydopamine (8 μg) was injected using a Hamilton syringe through one of the holes in a volume of 4 μl into the right medial forebrain bundle using the coordinates: A 3.4; V 3.1; and L 1.7 [23]. A second hole was drilled into the skulls of these animals for later injection of drugs into the striatum at the coordinates: A 8.2; V 0.0; and L 2.5. Twelve days after surgery the animals were tested for circling responses to apomorphine (1 mg/kg, s.c.). Only those animals that responded to apomorphine with contralateral turning were selected for studying the effects of drugs injected intrastriatally.

Fifteen days after 6-hydroxydopamine adminis-

Table 1. Effects of dopamine and amphetamine on the K^+-induced release of [^{3}H]acetylcholine from normal and reserpine α-methyl-*p*-tyrosine (α-MpT) pretreated striatal slices

Pretreatment	Drugs	N	Stimulated release (% of total tissue tritium)	% Control
Normal	Control	4	11.47 ± 0.45	100.0
	Amphetamine (30 μM)	4	5.69 ± 0.63*	47.3
Reserpine + αMpT	Control	3	12.14 ± 0.55	100.0
	Amphetamine (30 μM)	4	12.42 ± 0.89	102.3
Normal	Control	5	11.30 ± 0.46	100.0
	Dopamine (10 μM)	9	4.56 ± 0.55*	40.4
Reserpine + αMpT	Control	4	13.07 ± 1.49	100.0
	Dopamine (10 μM)	4	5.72 ± 0.39*	43.8

Each value is the mean ± SEM.
* $P < 0.025$ when compared to corresponding control value using the Mann–Whitney U test.

Table 2. Effect of sulpiride on the K^+-evoked release of [^{3}H]acetylcholine

Drugs	N	Basal release (% of tissue tritium content)	Stimulated release (% of tissue tritium content)
Control	20	2.81 ± 0.18	10.19 ± 0.71
Sulpiride (2 μM)	8	2.15 ± 0.21	9.43 ± 0.67

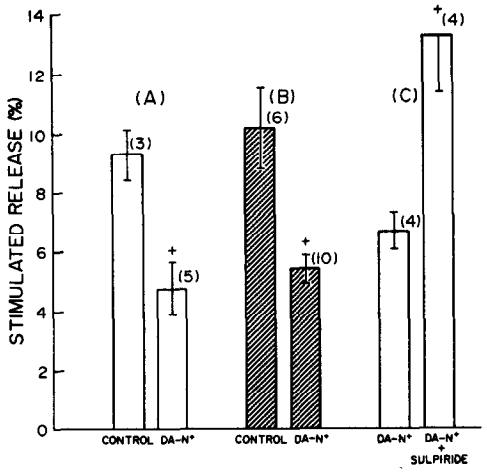

Fig. 4. Effect of the quaternary ammonium derivative of dopamine (DA-N^+) on the K^+-evoked release of [^{3}H]acetylcholine from striatal slices. The slices were incubated with [^{3}H]choline and then superfused. DA-N^+ (30 μM) and high K^+ medium (12.5 mM) were added at 40 and 50 min, respectively, after the onset of the superfusion. Stimulated release is the fractional release in the high K^+ medium above the spontaneous release. Study (A) refers to superfusion in normal medium; Study (B) refers to slices from reserpine plus α-methyl-*p*-tyrosine treated mice that were superfused with medium containing α-methyl-*p*-tyrosine (0.25 mM); Study (C) refers to the superfusion of slices in either normal medium or medium containing sulpiride (2 μM). Each value is the mean ± SEM, and the number of determinations is given in parentheses. Key: (+)$P < 0.025$ when compared to corresponding control value.

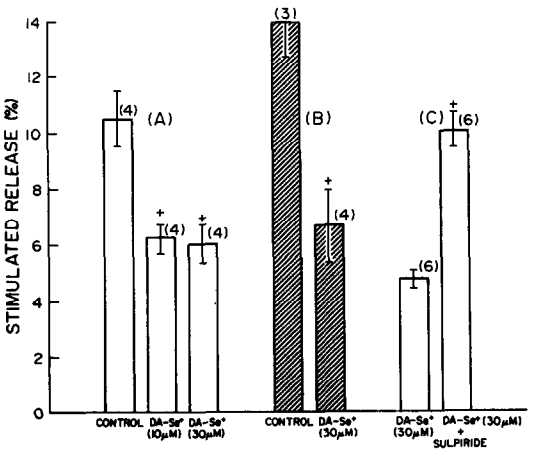

Fig. 5. Effect of the dimethylselenonium analog of dopamine (DA-Se^+) on the K^+-evoked release of [^{3}H]acetylcholine from striatal slices. The slices were incubated with [^{3}H]choline and then superfused. DA-Se^+ (30 μM) was added at 40 min, and the high K^+ was added to the medium at 50 min after the onset of the superfusion. Stimulated release is the fractional release in the presence of the high K^+ medium above the spontaneous release. Study (A) refers to slices superfused in normal medium; Study (B) refers to slices from animals treated with reserpine plus α-methyl-*p*-tyrosine that were superfused with medium containing α-methyl-*p*-tyrosine (0.25 mM); Study (C) refers to slices superfused in either normal medium or medium containing sulpiride (2 μM). Each value is the mean ± SEM, and the number of determinations is given in parentheses. Key: (+)$P < 0.025$ when compared to corresponding control value.

tration, rats were anesthetized with a halothane/oxygen mixture and placed in the stereotaxic frame. The needle of a Hamilton syringe was inserted into the hole in the skull above the striatum to the appropriate depth, and solutions of the drugs (dissolved in 0.2 mg/ml ascorbic acid, final pH 5.0) or vehicle were injected in a total volume of 2.0 μl at a rate of 1.0 μl/min. The rats recovered from this anesthesia within 5 min of drug injection and were placed in a glass chamber for the monitoring of circling behavior.

After the intrastriatal injections, the rats were placed in 22 liter hemispherical glass chambers. The number of complete turns (360°) *to the right or left* were counted for periods of 5 mins at 15-min intervals for 1 hr, starting at 15 min after drug injection. The results are expressed as the sum of complete turns during these intervals.

Statistics. Data are expressed as the mean ± SEM. Significant differences were evaluated using the nonparametric two-tailed Mann–Whitney U-test with a level of $P < 0.05$ being considered significant.

RESULTS

Effects of dopamine and amphetamine on the K^+*-evoked release of* [^{3}H]*acetylcholine from normal and dopamine-depleted slices (slices treated with reserpine and* α*-methyl-*p*-tyrosine).* The inhibitory effect of dopamine on the K^+-evoked release of [^{3}H]acetylcholine was dose-dependent, with a maximal inhibition of approximately 60% occurring at a concentration of 10 μM (Fig. 3). This inhibitory effect was still present in slices depleted of dopamine with reserpine and α-methyl-*p*-tyrosine (Table 1), but it was completely antagonized when sulpiride (2 μM), a dopamine receptor antagonist was added to the medium (data not shown). Sulpiride alone did not produce a significant change in the K^+-evoked tritium release (Table 2).

Amphetamine (30 μM), an indirectly acting dopaminergic agonist, also inhibited the K^+-evoked release of [^{3}H]acetylcholine from striatal slices (Table 1). However, in contrast to the inhibitory effect of dopamine, the effect of amphetamine was antagonized when reserpine plus α-methyl-*p*-tyrosine treated slices were used (Table 1).

Effect of the quaternary ammonium analog of dopamine on the K^+*-induced release of* [^{3}H]*acetylcholine from normal and dopamine-depleted slices.* The quaternary ammonium analog of dopamine (30 μM), which contains a permanent positively charged nitrogen (Fig. 1), inhibited the K^+-stimulated [^{3}H]acetylcholine release (Fig. 4). Although this inhibitory effect was antagonized by sulpiride (2 μM), it was not antagonized in slices treated with reserpine and α-methyl-*p*-tyrosine (Fig. 4).

Effect of the dimethylselenonium analog of dopamine on the K^+*-evoked release of dopamine from striatal slices.* The dimethylselenonium analog of dopamine, which contains a permanent positive charge (Fig. 1), also inhibited the K^+-stimulated release of [^{3}H]acetylcholine (Fig. 5). As with the quaternary ammonium analog, the inhibitory effect of the dimethylselenonium analog of dopamine was antagonized when sulpiride was added to the medium

Table 3. Effect of the monomethylsulfur analog of dopamine on the K^+-evoked release of [^{3}H]acetylcholine from striatal slices

Pretreatment	Drugs	Stimulated release (% of tissue tritium)
Normal	Control	13.7 ± 0.9
	Monomethylsulfur analog (30 μM)	11.3 ± 1.4
Reserpine + αMpT	Control	10.0 ± 0.7
	Monomethylsulfur analog (30 μM)	11.0 ± 0.8

* Each value is the mean ± SEM of three to four determinations.

but was still present in slices depleted of dopamine with reserpine and α-methyl-*p*-tyrosine (Fig. 5).

Effect of the uncharged methylsulfide analog of dopamine on the K^+-evoked release of [^{3}H]acetylcholine from striatal slices. To determine the effect of a permanently uncharged dopamine analog on the K^+-stimulated release of [^{3}H]acetylcholine, the monomethylsulfide analog of dopamine (Fig. 1) was synthesized and studied. This compound contains one less methyl group than the permanently charged dimethylsulfonium analog of dopamine, which has been shown previously to inhibit the K^+-evoked release of [^{3}H]acetylcholine [10]. As shown in Table 3, the methylsulfide analog produced a small inhibition of evoked [^{3}H]acetylcholine release, which was not statistically significant. This small degree of inhibition could be mediated by endogenous dopamine since this compound at a concentration of 30 μM produced approximately a 30% inhibition of [^{3}H]dopamine uptake into striatal slices (data not shown). The methylsulfide analog had no effect on evoked [^{3}H]acetylcholine release from slices depleted of dopamine with reserpine plus α-methyl-*p*-tyrosine (Table 3).

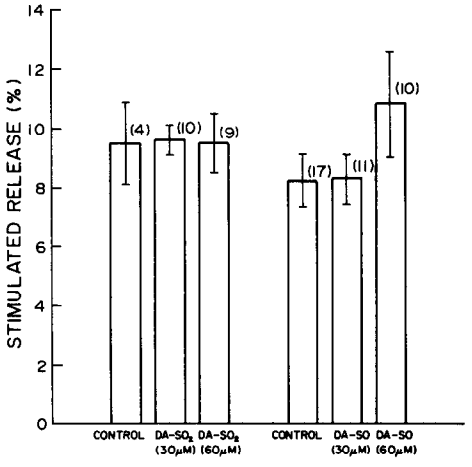

Fig. 6. Effects of the sulfoxide (DA-SO_2) and sulfone (DA-SO) analogs of dopamine on the K^+-evoked release of [^{3}H]acetylcholine from striatal slices. Slices were incubated with [^{3}H]choline and then superfused with normal medium. The test drugs were added at 40 min, and high K^+ medium was added at 50 min after the start of the superfusion. Each value is the mean ± SEM and the number of determinations is given in parentheses.

Effects of the sulfoxide and sulfone analogs of dopamine on the K^+-evoked release of [^{3}H]acetylcholine. The sulfoxide and sulfone analogs of DA do not contain a net positive charge (Fig. 1). As shown in Fig. 6, neither compound inhibited the K^+-evoked release of [^{3}H]acetylcholine at concentrations up to 60 μM.

Effect of atropine on the inhibition of the K^+-evoked [^{3}H]acetylcholine release from striatal slices induced by the permanently charged analogs of dopamine. To determine the role of muscarinic receptors in the inhibition of the evoked release of [^{3}H]acetylcholine by the charged dopamine analogs, the effects of these analogs were determined after atropine, a muscarinic receptor antagonist, was added to the medium. Atropine at a concentration

Table 4. Effect of atropine on the inhibition of evoked [^{3}H]acetylcholine release induced by the permanently charged analogs of dopamine and methacholine

Treatment	Stimulated release (% of tissue tritium)	
	High K^+ medium	High K^+ medium with atropine (0.1 μM)
Control	12.88 ± 0.63 (18)	13.93 ± 0.75 (6)
Methacholine (100 μM)	7.42 ± 0.69* (6)	13.12 ± 0.95 (6)
Quaternary ammonium analog (30 μM)	6.70 ± 0.91* (4)	7.28 ± 0.64† (4)
Selenonium analog (30 μM)	6.17 ± 0.63* (8)	6.35 ± 0.57† (7)
Sulfonium analog (30 μM)	4.48 ± 0.35* (3)	4.90 ± 0.80† (2)

All drugs were present in the high potassium medium. Each value is the mean ± SEM, and the number of determinations is given in parentheses.

* $P < 0.05$ when compared to control.

† $P < 0.05$ when compared to control in medium with atropine.

of 0.1 μM antagonized the inhibition of the K^+-evoked [^{3}H]acetylcholine release induced by 100 μM methacholine (Table 4), whereas sulpiride (2 μM) did not alter the response to methacholine (data not shown). These observations indicate that the methacholine-induced response was mediated through the activation of muscarinic but not dopaminergic receptors. In contrast, atropine (0.1 μM) did not alter the inhibition of evoked [^{3}H]acetylcholine release induced by any of the permanently charged analogs (Table 4). These results suggest that the inhibition of evoked [^{3}H]acetylcholine induced by the permanently charged analogs of dopamine is not mediated by the activation of muscarinic receptors.

Effect of the unilateral injection of dopamine agonists into striatum of rats pretreated with 6-hydroxydopamine. Rats were injected in the right medial forebrain bundle with 6-hydroxydopamine as described in Materials and Methods. Two to three weeks after injection, the rats were anesthetized with halothane and injected with the potential dopamine agonists into the denervated striatum (drug injection on the same side of the brain as the 6-hydroxydopamine injection). Circling behavior was then recorded at 5-min intervals at 15, 30, 45, and 60 min after injection. Under these conditions, the intrastriatal administration of dopamine has been shown previously to produce a marked stimulation of contralateral circling [9, 22]. The rotational behavior produced by amphetamine, however, was not significantly different from that of saline (Table 5). The permanently charged quaternary ammonium analog and the dimethylsulfonium analog of dopamine at the same dose as amphetamine elicited significant contralateral circling (Table 5). In contrast, the permanently uncharged monomethylsulfide analog of dopamine did not elicit significant contralateral circling. Thus, the effectiveness of these compounds on the dopaminergic receptor mediating circling behavior was similar to that regulating the depolarization-evoked release of [^{3}H]acetylcholine.

Table 5. Effect of the direct intrastriatal injection of the charged and uncharged sulfur analog of DA, the quaternary ammonium derivative of DA, and amphetamine on the circling behavior of rats previously lesioned unilaterally with 6-hydroxydopamine

Drug	Turns*
Control	4.2 ± 1.0
Amphetamine	9.0 ± 4.7
Quaternary ammonium analog	122.0 ± 36.9†
Charged dimethylsulfonium analog of DA	181.2 ± 41.7†
Uncharged methylsulfide analog of DA	0.3 ± 0.1

* The number of turns in the ipsilateral and contralateral direction were determined at 5-min intervals at 15, 30, 45 and 60 min. The net number of turns for each animal (contralateral minus ipsilateral) was added together. The dose for all drugs was 100 nmol injected in 2.0 μl. Controls were injected with 2 μl saline. Each value is the mean ± SEM of four animals. All of the mean values refer to net circling in the contralateral direction.

† $P < 0.05$ when compared to controls.

DISCUSSION

The results of this study show that permanently charged analogs of dopamine of different chemical structures were able to inhibit the K^+-stimulated release of [^{3}H]acetylcholine from striatal slices. This inhibition could be reversed by the addition of sulpiride, a dopamine receptor antagonist, to the medium but it was not reversed by atropine (0.1 μM) which completely antagonized the inhibition of evoked [^{3}H]acetylcholine release induced by methacholine, a muscarinic agonist. Therefore, these studies provide support for the concept that the charged molecular form of a dopamine agonist can activate dopaminergic receptors.

The permanently charged dimethylselenonium and quaternary ammonium analogs of dopamine could inhibit the evoked [^{3}H]acetylcholine release by acting directly on the dopaminergic receptor or indirectly by enhancing the synaptic activity of endogenous dopamine, i.e. by releasing or blocking the reuptake of endogenous dopamine [24]. To distinguish between these possibilities, the effects of the charged analogs were studied under conditions of dopamine depletion with reserpine and α-methyl-*p*-tyrosine. These drugs were found to antagonize the inhibition of the K^+-evoked release of [^{3}H]acetylcholine induced by amphetamine, an indirectly acting amine, but not that induced by dopamine. Under these same conditions, the charged dopamine analogs were also able to inhibit the evoked release of [^{3}H]acetylcholine. In addition, after intrastriatal injection to 6-hydroxydopamine-pretreated rats, the charged analogs were able to induce contralateral circling, whereas an equimolar dose of amphetamine was ineffective. These observations suggest that the effects of the permanently charged dopamine analogs are not mediated by endogenous dopamine but are due to a direct action on dopaminergic receptors.

The demonstration that a permanently charged analog of dopamine can activate dopaminergic receptors does not indicate whether a charge on the molecule is *required* for agonist activity. To study this problem, we synthesized and determined the effects of analogs of dopamine that have no net charge in physiological solution. It was found that neither the sulfoxide nor the sulfone analogs of dopamine produced an inhibitory effect on the K^+-evoked release of [^{3}H]acetylcholine from striatal slices at concentrations up to 60 μM. Although the permanently uncharged monomethylsulfide analog of dopamine did produce a small but not significant inhibitory effect (−18%) on the K^+-evoked release of [^{3}H]acetylcholine, the inhibition may have been mediated by the synaptic accumulation of endogenous dopamine, since the compound was found to inhibit the uptake of [^{3}H]dopamine into striatal slices (data not shown). In support of this idea, the monomethylsulfide analog did not inhibit the evoked release of [^{3}H]acetylcholine in slices in which dopamine was depleted with reserpine and α-methyl-*p*-tyrosine. The ineffectiveness of the permanently uncharged analogs of dopamine in activating dopaminergic receptors in this *in vitro* model is consistent with results obtained in an *in vivo* model in which

dopaminergic agonists were injected unilaterally into the striatum of rats on the same side of the brain in which 6-hydroxydopamine was previously injected into the medial forebrain bundle. Although the intrastriatal injections of the permanently charged dimethylsulfonium and quaternary ammonium analogs of dopamine elicited contralateral circling, the permanently uncharged monomethylsulfide analog, like amphetamine, did not produce significant circling behavior. The results of these studies suggest that a charge on a dopamine agonist molecule is required for the activation of dopamine receptors.

The dopamine receptor that regulates the depolarization-induced release of [^{3}H]acetylcholine seems to be a receptor of the D-2 class. Thus, bromocriptine, which does not appear to activate the D-1 receptors associated with adenylate cyclase in striatal homogenates [2, 3], can inhibit the depolarization-evoked release of [^{3}H]acetylcholine [25]. In contrast, SKF-38393, which selectively activates the D-1 dopaminergic receptor, has no inhibitory activity on this system [26]. In addition, the inhibition of the depolarization-induced [^{3}H]acetylcholine release by dopaminergic agonists is antagonized by metoclopramide and sulpiride [26], which are specific D-2 receptor antagonists [27, 28]. The present study, therefore, indicates that agonists of the D-2 dopaminergic receptor subtype must activate the receptor in the charged form. However, it does not provide information on the molecular requirements for activation of the D-1 receptor.

The results of this and a previous study [10] indicate that dopamine agonist activity can be produced when each of three different atoms is present on the α-carbon of the side chain of dopamine. Thus, dopamine agonist activity was obtained when a charged nitrogen (quaternary ammonium group), selenium (dimethylselenonium group), or sulfur (dimethylsulfonium group) atom was present at this site. Therefore, these results suggest that the type of atom on the α-carbon of dopamine may be less important to dopamine agonist activity than the ability of this atom to become positively charged in aqueous solution.

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