Dopaminergic Agonists: Comparative Actions of Amine and Sulfonium Analogues of Dopamine

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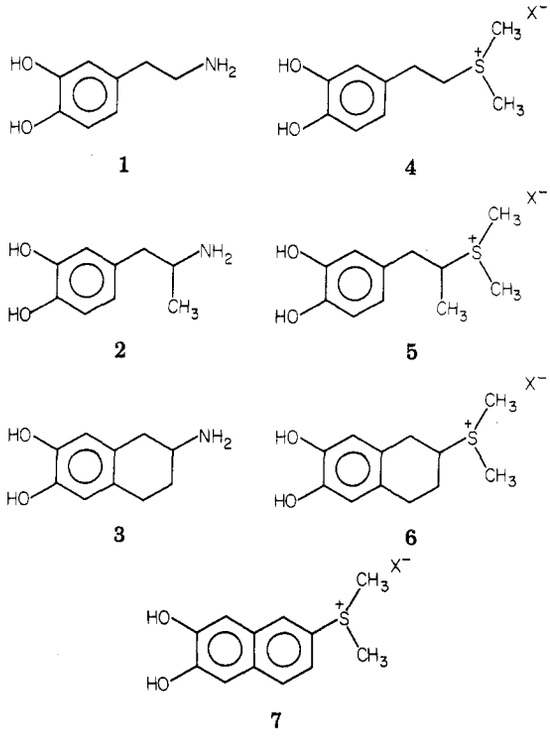
We have investigated the possibility that structural modifications of the sulfonium analogue of dopamine (4) would produce the same pattern of biological activity as structural modifications of dopamine. A series of methyl- tetralinyl-, and naphthalenylsulfonium analogues **5–7** were prepared and tested for their ability to inhibit the potassium-evoked release of [^{3}H]acetylcholine from striatal slices. All compounds were tested under normal conditions and after depletion of dopamine stores with reserpine and α-methyl-*p*-tyrosine. The amine and sulfonium analogues **2–6** all showed direct agonist activity. The sulfonium analogue **7** produced, predominantly, indirect activity. In contrast to the amine analogues, chemical modifications of the sulfonium compounds produced little change in their dopamine agonist activity.

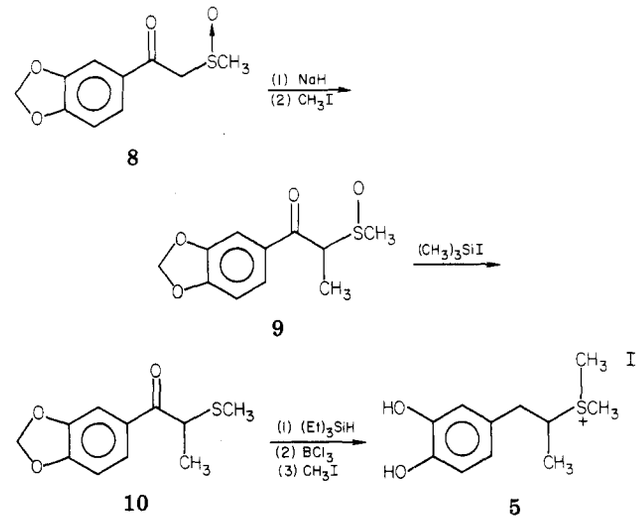
Structural requirements of compounds possessing dopaminergic activity have been a subject of considerable study.[1-4] We have recently reported that the nitrogen atom of dopamine (1) is not essential for dopaminergic



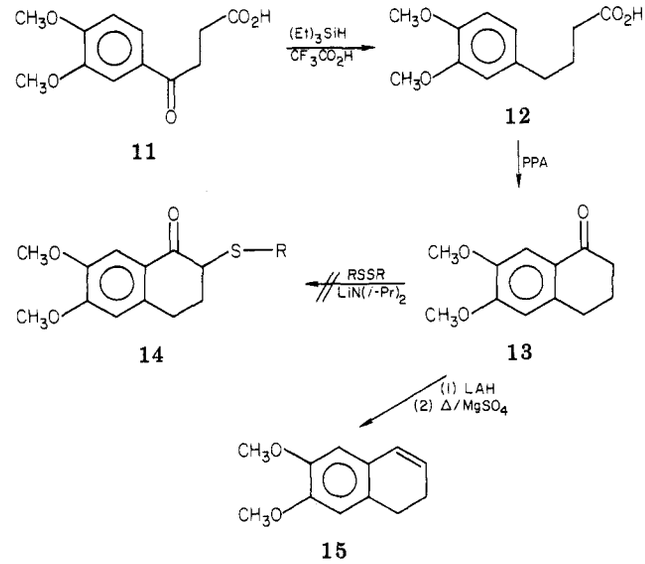
agonist activity and that sulfonium analogues of dopamine, e.g., 4, possess significant activity in both dopamine binding and behavioral studies.[5]

An important approach toward understanding the conformational requirements for drugs acting on dopamine receptors has been to examine molecules possessing a dopamine segment in structures of conformationally restricted molecules, such as 6,7-dihydroxy-2-aminotetralin (ADTN, 3). The synthesis of ADTN was reported by Thrift in 1967,[6] and it was later shown to have dopaminergic activity by Miller et al.[7] and Woodruff.[8] It was then

Scheme I



Scheme II



proposed by Cannon[1] that ADTN could be considered a β rotameric form of dopamine, and when compared to α-methyldopamine (2), it was found that ADTN was more potent. In order to compare an analogous series of sulfonium analogues with those of dopamine, we prepared compounds **4–6**. We have also included in our study the naphthalene derivative **7**, which could be prepared easily from one of the intermediates in the synthesis of **6**. The compounds (1–7) were examined for their ability to inhibit, via stimulation of dopamine receptors, the in vitro po-

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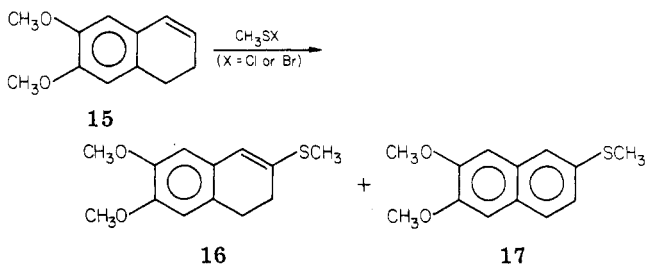
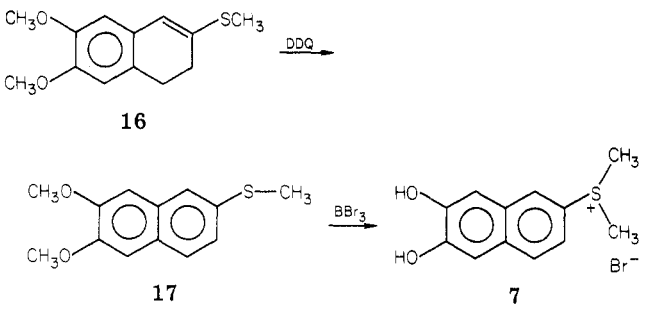
Scheme III



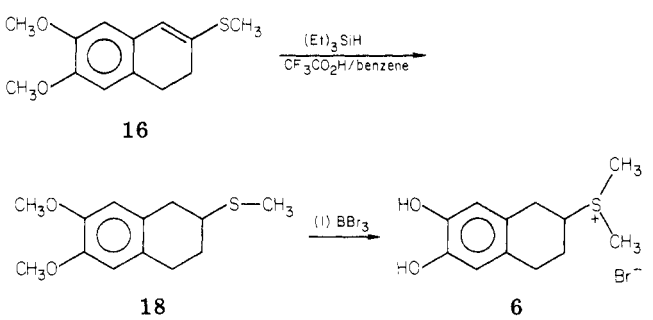
Scheme IV



tassium (K^+) induced release of acetylcholine (Ach) in the rat caudate nucleus.

Chemistry. We have previously reported the synthesis of **4**.[5] An important intermediate in the synthesis of **4** is the keto sulfoxide **8**, which was methylated to give **9** and then converted to **5** (Scheme I) in a sequence similar to that reported for the preparation **4**.[5] Our initial attempt at preparing the tetralinylsulfonium analogue **6** is shown in Scheme II. We prepared 3-(3,4-dimethoxybenzoyl)-propionic acid (**11**) as described by Adams et al.[9] Reduction of **11** with triethylsilane in trifluoroacetic acid[10] gave a 94% yield of **12**. Tetralone **13**[11] was obtained, by treating **12** with polyphosphoric acid, in 74% yield. We attempted to prepare **14** according to the procedures of Trost et al.[12] by treatment of **13** with lithium diisopropyl amide and methyl disulfide but were unable to isolate any of the desired **14**. However, treatment of **15** with methylsulfenyl chloride, prepared from dimethyl sulfide and sulfuryl chloride, provided a mixture of the dihydronaphthalene **16** and the naphthalene **17** (Scheme III). The use of methylsulfenyl bromide tended to give a higher yield of **17**. The mixture of **16** and **17** could be separated by flash chromatography, and pure **16** could be converted to **17** by DDQ (Scheme IV). Treatment of **17** with BBr_3 gave **7** in one step (61% yield). The reduction of **16** was carried out with triethylsilane in trifluoroacetic acid and benzene to give **18** in 93% yield (Scheme V). The use of benzene as a solvent provided the best results, since if only trifluoroacetic acid was used as a solvent in the reaction, a black tar was obtained. Initially, we attempted to demethylate **18** with BBr_3 and then treat it with methyl iodide to form a sulfonium iodide salt of the catechol **6**, but we always obtained a mixture of the iodide and bromide salts. We found it much easier to treat **18** with BBr_3 and in one step isolate the pure sulfonium bromide salt **6**.

Scheme V



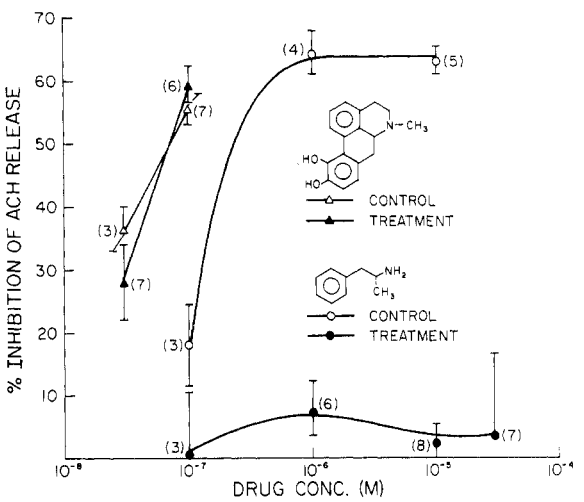


Figure 1. Effect of reserpine and α-MPT on the inhibition of [^{3}H]Ach release produced by amphetamine and apomorphine. Striatal slices were prepared from either control mice or mice treated with reserpine (5 mg/kg, ip, 20 h before killing) and α-MPT (250 mg/kg, ip, 1.5 h before killing). The slices were incubated with [^{3}H]choline (0.1 μm), washed, and superfused with physiological medium. The medium used for superfusion of the treated slices contained α-MPT (250 μM). [^{3}H]Ach release was evoked by two successive exposures (S_1 and S_2) to 12.5 mM K^+ at 55 and 90 min after the start of the superfusion. Apomorphine and amphetamine were added 20 min before S_2. The drug effects are expressed as a percentage of the S_2/S_1 ratio obtained from slices superfused with medium that contained no dopaminergic agonist. The S_2/S_1 ratio from control slices was 0.92 ± 0.01 (SEM); the S_2/S_1 ratio from slices treated with reserpine and α-MPT was 1.01 ± 0.03 (SEM). Each value is the mean plus or minus SEM, and the number of observations is given in parentheses.

Effect of Apomorphine and Amphetamine on the Evoked Release of [^{3}H]Ach from Striatal Slices. Mice were pretreated with reserpine (5 mg/kg ip) and α-methyl-*p*-tyrosine (α-MPT; 250 mg/kg, ip) at 20 and 1.5 h, respectively, before killing. Striatal slices were prepared from these pretreated mice or control mice and incubated with [^{3}H]choline (0.1 μM) for 20 min. The slices from the drug-pretreated mice were then superfused with medium containing α-MPT (250 μM), while the slices from controls were superfused with normal medium. [^{3}H]Ach release was evoked by two successive exposures to K^+ at 55 (S_1) and 90 (S_2) min after the onset of the superfusion. Apomorphine and amphetamine were added to the superfusion medium 20 min before S_2. Figure 1 shows that both amphetamine and apomorphine inhibited the K^+-evoked release of [^{3}H]Ach from control slices. However, the effects of apomorphine and amphetamine on [^{3}H]Ach release were different in the slices treated with reserpine and α-MPT. Under these conditions, the inhibitory effect of amphetamine was blocked, while the inhibitory effect of apomorphine was similar to that in control slices.

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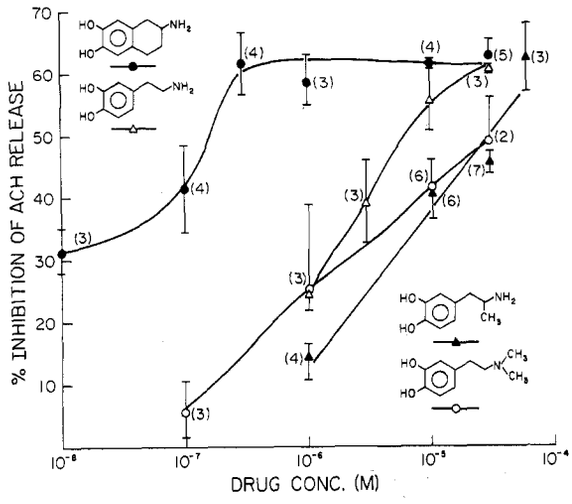

Figure 2. Effect of nitrogen-containing dopaminergic agonists on the K^+-induced release [^{3}H]Ach from striatal slices. Striatal slices were incubated with [^{3}H]choline (0.1 μM) and then superfused with physiological medium. [^{3}H]Ach release was evoked by two successive exposures (S_1 and S_2) to K^+ at 55 and 90 min after the start of the superfusion. The drugs were added 20 min before S_2. The results are expressed as the percentage inhibition of the control S_2/S_1 ratio produced by the drugs. The control S_2/S_1 ratio was 0.92 ± 0.01 (SEM). Each value is the mean plus or minus SEM, and the number of determinations is given in the parentheses.

Effect of Dopaminergic Agonists on the K^+-Evoked Release of [^{3}H]Ach. All of the dopaminergic agonists studied inhibited the K^+-evoked release of [^{3}H]Ach. However, there was a marked difference in potency between these drugs (Figure 2). Thus, of the drugs tested, 6,7-ADTN was the most potent, with the concentration that inhibited [^{3}H]Ach release by 50% (IC_{50}) being approximately 0.01 μM. In contrast, the approximate IC_{50} for dopamine, dimethyldopamine, and α-methyldopamine was between 1 and 10 μM, with dopamine being the most potent of these latter compounds. This order of potency was not changed when the dopaminergic agonists were tested on reserpine–α-MPT treated slices (Figure 3).

Effect of the Sulfonium Analogues of Dopamine on the Evoked Release of [^{3}H]Ach from Striatal Slices. Figure 4 shows that all of the sulfonium analogues of dopamine tested inhibited the evoked release of [^{3}H]Ach. Unlike the effect produced by the nitrogen-containing dopamine agonists, all of the sulfonium analogues showed a similar inhibitory potency. Thus, the inhibitory effect of **6**, the sulfonium analogue of 6,7-ADTN, was approximately the same as that of **3**, the sulfonium analogue of dopamine. When the slices were prepared from mice treated with reserpine–α-MPT, most of the sulfonium analogues were still able to inhibit the K^+-evoked [^{3}H]Ach release, although there was a decrease in their effectiveness (Figure 5). However, the inhibitory effect of **7** was reduced (Table I) by reserpine and α-MPT treatment.

Discussion

The potassium-induced release of [^{3}H]Ach in striatal slices is a useful model in which to evaluate the dopamine agonist activity of sulfonium analogues. Dopamine agonists have been shown to decrease the depolarization-induced release of [^{3}H]Ach from striatal slices.[13,14] The

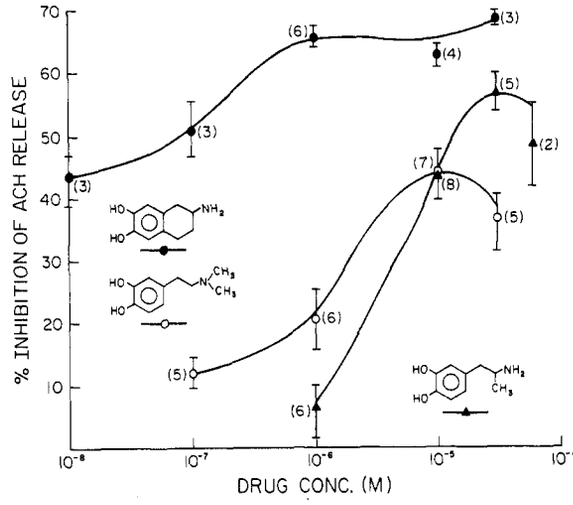

Figure 3. Effect of reserpine and α-MPT pretreatment on the inhibition of K^+-induced [^{3}H]Ach release by the nitrogen-containing dopaminergic agonist. Striatal slices were prepared from control mice or mice injected with reserpine (5 mg/kg ip) and α-MPT (250 mg/kg, ip) 20 and 1.5 h before killing, respectively. The slices were incubated with [^{3}H]choline (0.1 μM), washed, and then superfused. Control slices were superfused with normal media while slices from the treated animals were superfused with medium containing α-MPT (250 μM). [^{3}H]Ach release was evoked by two successive exposures (S_1 and S_2) to K^+ at 55 and 90 min after the start of superfusion. Drugs were added 20 min before S_2. The results are expressed as the percentage inhibition of the S_2/S_1 ratio determined when no drugs were added to the medium, and this value was 0.98 ± 0.02. Each value is the mean plus or minus SEM, and the number of observations is given in the parentheses.

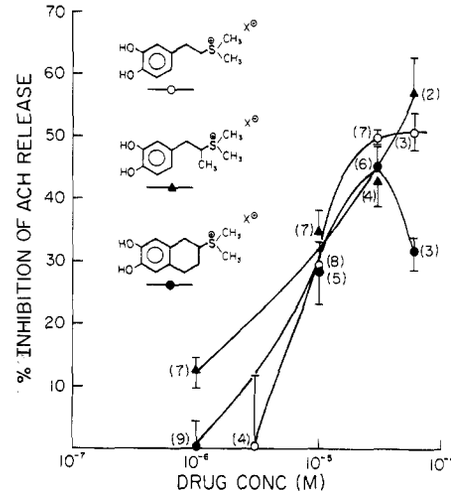

Figure 4. Effect of the sulfonium analogues of DA on the K^+-induced release of [^{3}H]Ach from striatal slices. The slices were incubated with [^{3}H]choline (0.1 μM) and then superfused with physiological medium. [^{3}H]Ach release was evoked by two successive exposures (S_1 and S_2) to K^+ at 55 and 90 min after the start of the superfusion. The sulfonium analogues were added 20 min before S_2. The results are expressed as the percentage inhibition of the control S_2/S_1 ratio produced by the drugs. The control S_2/S_1 ratio was 0.92 ± 0.03 (SEM). Each value is the mean plus or minus SEM, and the number of determinations is given in the parentheses.

present study shows that both amphetamine and apomorphine inhibited the release of [^{3}H]Ach from striatal slices (Figure 1). However, the action of amphetamine in this system was inhibited if the slices were treated with reserpine and α-MPT, while this treatment did not affect the action of apomorphine, a direct-acting agonist. Thus, reserpine and α-MPT treatment can be used to distinguish

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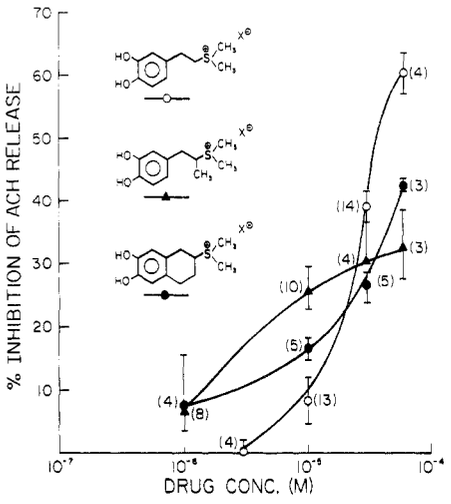

Figure 5. Effect of reserpine and α-MPT pretreatment on the inhibition of K^+-induced [^{3}H]Ach release by the sulfonium analogues of DA. The design of these experiments was the same as that described in Figures 1 and 3. The control S_2/S_1 ratio was 0.99 ± 0.03 (SEM). Each value is the mean plus or minus SEM, and the number of determinations is given in parentheses.

Table I. Effects of the Naphthalenylsulfonium Analogue (7) of DA on the K^+-Evoked Release of [^{3}H]Ach[a]

concn, μM	control: S_2/S_1	% zero concn	reserpine–α-MPT: S_2/S_1	% zero concn
0.0	0.92 ± 0.02 (11)	100 ± 3	0.98 ± 0.03 (9)	100 ± 3
0.3	0.89 ± 0.04 (2)	95 ± 4		
1.0	0.83 ± 0.05 (6)	90 ± 5	0.85 ± 0.06 (4)	87 ± 6
3.0	0.68 ± 0.02 (3)[b]	75 ± 2	0.81 ± 0.06 (8)	83 ± 6
10.0	0.49 ± 0.03 (4)[b]	53 ± 3	0.86 ± 0.05 (8)	88 ± 5

[a] Striatal slices prepared from control or reserpine–α-MPT treated mice were incubated with [^{3}H]choline, washed, and superfused. The slices from controls were superfused in normal medium, while those from treated animals were superfused in medium containing α-MPT (250 μM). [^{3}H]Ach release was evoked by two successive exposures (S_1 and S_2) to K^+ at 55 and 90 min after the start of the superfusion. Drugs were added 20 min before S_2. The results are expressed as the percentage inhibition of the S_2/S_1 ratio determined in the absence of drugs. Each value is the mean plus or minus standard error of the mean with the number of determinations given in the parentheses. [b] $p < 0.05$ (Mann Whitney U test).

between a direct- and indirect-acting dopamine agonist.

The object of this study was to determine the effect of structural changes in the sulfonium analogue molecule on dopamine agonist activity and to compare the changes observed in biological activity with those for dopamine and closely related analogues. The present study shows that ADTN, dopamine, *N*,*N*-dimethyldopamine, and α-methyldopamine all inhibited [^{3}H]acetylcholine release from striatal slices. However, these compounds showed a marked difference in potency. ADTN (Figure 2) and apomorphine (Figure 1) were found to be approximately 30 times more potent than the other compounds tested. The order of potency of the remaining compounds was as follows: dopamine > *N*,*N*-dimethyldopamine ≈ α-methyldopamine. The differential potencies of these compounds do not appear to be related to their ability to release endogenous dopamine from presynaptic nerve terminals, since the relative potencies of these dopamine agonists were not changed by reserpine–α-MPT treatment. The higher potency of ADTN and apomorphine in this system suggests that the receptors involved in the inhibition of [^{3}H]Ach release are particularly sensitive to drugs in which the side chain is conformationally restricted and planar. This concept has been previously[15] used to explain the low level of dopaminergic activity of α-methyldopamine and analogues, since the catechol ring of these compounds are not coplanar with respect to the β-phenethylamine portion of the dopamine segment of the molecule.

The sulfonium analogues that were tested, like the amine analogues of DA, also inhibited K^+-induced [^{3}H]Ach release from striatal slices. This inhibition seems to be due primarily to a direct agonist action, since their inhibitory effects on release were not blocked by reserpine–α-MPT treatment, although some inhibitory effect was noted. In contrast to the methyl- and tetralinylsulfonium compounds, the inhibitory effect of the naphthalenylsulfonium analogue (7) was markedly inhibited by reserpine–α-MPT treatment, suggesting a predominantly indirect action for this compound. Surprisingly, the biological activity of the sulfonium analogues of dopamine did not show large differences with the structural modifications. Thus, the potency of the ADTN-sulfonium analogue was similar to the sulfonium analogue of dopamine and α-methyldopamine, in contrast to what was observed with ADTN, dopamine, and α-methyldopamine. These observations show that there are significant differences in the structure–activity relationships between sulfonium and amine analogues of dopamine.

These studies show that the changes in molecular structure that alter the potency of amine dopaminergic agonists do not apply to the sulfonium analogues in the inhibition of K^+-induced [^{3}H]Ach release from striatal slices. It is possible that the permanent charge of the sulfonium analogues may minimize the role played by other structural features ordinarily deemed important for dopaminergic activity.

Experimental Section

Melting points (uncorrected) were determined on a Thomas-Hoover melting point apparatus. Spectral data were obtained with a Beckman 4230 infrared spectrophotometer and a Varian A-60A NMR (60 MHz) or a Bruker HX-90E NMR spectrometer (90 MHz) in pulse mode. Mass spectra were obtained with a DuPont Model 21-491 double-focusing mass spectrometer. Analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN. Analytical results for elements indicated were within ±0.4% of the theoretical values.

2-(Methylsulfinyl)-3′,4′-(methylenedioxy)propiophenone (9). A 50% oil dispersion of NaH was washed with hexane to remove the oil and dried under vacuum overnight at room temperature. This sodium hydride (150 mg, 6.25 mmol) was added to 20 mL of dry Me_2SO at room temperature and stirred under argon for 30 min. To this was added 3′,4′-(methylenedioxy)-2-(methylsulfinyl)acetophenone[5] (1.13 g, 5 mmol) dissolved in 10 mL of Me_2SO, and this mixture was stirred at room temperature for 30 min. To the mixture was added dry methyl iodide (0.32 mL) by syringe, and the mixture was stirred at room temperature under argon for an additional 30 min. The reaction mixture was poured into 50 mL of saturated NH_4Cl aqueous solution, extracted with CH_2Cl_2 (3 × 50 mL), washed with saturated NH_4Cl aqueous solution and then twice with H_2O, and dried over $MgSO_4$. Removal of the CH_2Cl_2 afforded a yellow oil, which was chromatographed on silica gel with acetone–CH_2Cl_2 (1:4) for purification to yield 0.95 g (79%) of a yellow oil containing a mixture of the diastereoisomers of **9**: NMR ($CDCl_3$, 90 MHz) δ 7.70–7.45 (m, 2 H, Ar H), 6.90, (d, J = 8.3 Hz, 1 H, Ar H), 6.07 and 6.08 (2 s, 2 H, OCH_2O), 4.80 and 4.56 (2 q, J = 7 Hz, CH), 2.50 and 2.45 (2 s, 3 H, SCH_3), 1.59 and 1.57 (2 d, J = 7 Hz, 3 H, CCH_3); mass spectrum, m/e 240.04. Anal. ($C_{11}H_{12}SO_4 \cdot {}^1/_6H_2O$) C, H, S.

2-(Methylthio)-3′,4′-(methylenedioxy)propiophenone (10). To a 100-mL flask was added **9** (0.78 g, 3.25 mmol) and NaI (1.97

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g, 13.1 mmol); this mixture was dissolved in 20 mL of CH_3CN and then stirred at room temperature and under argon. Trimethylchlorosilane (1 mL, 0.856 g, 7.96 mmol) was added to the mixture dropwise by a syringe. The reaction mixture was stirred under argon at room temperature for 3 h and to this was added 50 mL of a 10% NaS_2O_3 aqueous solution. The reaction mixture was then extracted with CH_2Cl_2 (3 × 75 mL); the combined organic phase was washed with saturated NaCl solution. After removal of the solvent, the reaction mixture was placed on a silica gel column and eluted with CH_2Cl_2 to yield **10** (0.62 g, 85%) as a yellow oil: NMR ($CDCl_3$, 90 MHz) 7.7–7.4 (m, 2 H), 6.75 (d, J = 9 Hz, 1 H), 6.05 (s, 2 H), 4.24 (q, J = 7.5 Hz, 1 H), 1.97 (s, 3 H), 1.52 (d, J = 7.5 Hz, 3 H). Anal. ($C_{11}H_{12}SO_3$) C, H, S.

[1-(3,4-Dihydroxyphenyl)-2-propyl]dimethylsulfonium Iodide (5). To a 100-mL flask containing **10** (0.62 g, 2.77 mmol) were added 10 mL of freshly distilled CF_3CO_2H under argon with stirring at room temperature and 2 mL of Et_3SiH (11.7 mmol) dropwise with a syringe, and the mixture was stirred at room temperature under argon for 4 h. Water (50 mL) was added, and then the mixture was extracted with ether. The combined ether solution was washed with 15% KOH and H_2O and then dried over $MgSO_4$. Removal of the solvent gave a yellow oil; the oil was placed on a silica gel column and eluted with CH_2Cl_2/hexane (1:1) to yield a pale yellow oil (0.44 g, 76%): NMR ($CDCl_3$, 90 MHz) 6.80–6.67 (m, 3 H), 5.93 (s, 2 H), 2.94–2.35 (m, 3 H, Ar CH_2 and CH), 2.09 (s, 3 H), 1.23 (d, J = 7 Hz).

Removal of the methylenedioxy protecting group was carried out under anhydrous conditions. To a solution of the methylenedioxy derivative (40 mg, 0.19 mmol) in 2 mL of CH_2Cl_2, cooled to −78 °C and stirred under argon was added by a syringe 0.5 mL of 1 M BCl_3/hexane solution. The temperature was gradually raised to room temperature, and stirring was continued under argon for 3 h. Dry MeOH (10 mL) was added dropwise, to react with excess BCl_3. After the methanol-treated mixture was stirred at room temperature for 3 h, the solvents were removed under vacuum at 50–60 °C for 1 h. Excess freshly distilled MeI was added, and the mixture was stored for 3 days in the freezer to give white crystals, which were washed with CH_2Cl_2 and dried by vacuum pump to yield 65 mg (quantative yield) of a white solid: mp 101–103 °C; NMR (Me_2SO-d_6, 90 MHz) δ 8.95, 8.87 (2 s, 2 H), 6.74–6.48 (m, 3 H), 3.60–3.42 (m, 1 H), 2.72–2.49 [m, 8 H, Ar CH_2, and $S(CH_3)_2^+$], 2.00 (d, J = 8.9 Hz, 3 H). Anal. ($C_{11}H_{17}SO_2I$) C, H, S.

3-(3,4-Dimethoxybenzoyl)propionic Acid (11). To a mixture of 66 g of succinic anhydride, 96 g of veratrol, and 600 mL of nitrobenzene, cooled in an ice bath and efficiently stirred, was added 180 g of aluminum chloride at such a rate that the temperature remained below 25 °C. The reaction mixture was allowed to come slowly to room temperature and stirred for 12 h. It was then poured into a mixture of 300 mL of concentrated HCl and 700 mL of ice–water and allowed to stand for 2 h, and the product was filtered. It was purified by dissolving in 1.0 L of 1 N NaOH, washing with 500 mL of ether, *treating with Norit*, and acidifying with concentrated HCl. Crystallization from boiling water gave 112 g (69%) of colorless crystals, mp 158–160 °C.[16]

4-(3,4-Dimethoxyphenyl)butanoic Acid (12). To a solution of 3-(3,4-dimethoxybenzoyl)propionic acid (3.6 g) in CF_3CO_2H (25 mL) was added dropwise EtSiH (7 mL),[10] and the resulting mixture was stirred vigorously for 6 h. Removing the solvent in vacuo gave an oily residue, which was dissolved in ether (50 mL), washed with water (50 mL), and extracted with $NaHCO_3$ (3 g in 100 mL). The ether layer was dried on anhydrous $MgSO_4$ and evaporated to afford a white solid, which was recrystallized from ether–*n*-hexane to yield 3.2 g (94%) of acid **12**, mp 60–61 °C.[17]

6,7-Dimethoxy-1-tetralone (13). The butanoic acid **12** (2.0 g) was added in small portions with stirring to 25 g of PPA at 75 °C. After 15 min of heating and stirring, an additional 15 g of PPA was added, and stirring and heating were continued for 1 h. To the resulting deep-red mixture was added 120 mL of ice–water, and the aqueous mixture was extracted with 50 mL of CH_2Cl_2. The organic layer was washed with 50 mL of H_2O and then with 50 mL of saturated $NaHCO_3$, dried ($MgSO_4$), and treated with a small amount of active charcoal. Evaporating the solvent gave a colorless solid, which was recrystallized from *n*-hexane/CH_2Cl_2 to afford 1.37 g (74.5%) of colorless crystalline tetralone **13**, mp 96–98 °C.[18]

6,7-Dimethoxy-1,2-dihydronaphthalene (15). A solution of the tetralone **13** (6.0 g) in anhydrous THF (30 mL) was added dropwise with stirring to an ice-cold suspension of LAH (1.0 g) in anhydrous THF (30 mL). After the mixture was stirred for 3 h at room temperature, excess LAH was decomposed by the addition of a small amount of ethyl acetate; water was then added, and the THF layer was separated. The aqueous layer was extracted with ether, and the organic layers were dried ($MgSO_4$). The organic layer was evaporated to give a crude tetralol, which was dissolved in ether (300 mL) and passed through a short silica gel column to afford 5.8 g of pure oil tetralol (96%). Distillation caused dehydration to yield dihydronaphthalene (**15**). An alternate way of preparing **15** was to heat to reflux for 1 h with stirring a mixture of tetralol (5.5 g) in dry benzene (100 mL) and anhydrous $MgSO_4$ (10 g). The resulting mixture was passed through the short silica gel column with ether–*n*-hexane (1:1) to yield 4.95 g of pure dihydronaphthalene [98.5%; bp 122 °C (0.1 mm)].[19] Compound **15** solidified, and it was recrystallized from pentane to give colorless crystals, mp 40.0–41.5 °C.

6,7-Dimethoxy-3-(methylthio)-1,2-dihydronaphthalene (16). To a mixture of dihydronaphthalene (0.95 g), anhydrous $NaHCO_3$ (0.5 g), and anhydrous benzene (10 mL) was added dropwise CH_3SCl, prepared from $(CH_3S)_2$ (0.2 g) and SO_2Cl_2 (0.27 g), in anhydrous benzene (2 mL) with stirring at room temperature. After the mixture was stirred for 10 min, 10 mL of water was added, and the organic layer was treated by flash chromatography on silica gel with petroleum ether–ethyl acetate (9:1) to yield trace amount of (methylthio)naphthalene **17** and 602 mg (64%) of (methylthio)dihydronaphthalene **16**: mp 77.5–78.5 °C (from ether–pentane): NMR ($CDCl_3$) δ 6.68 (s, 1 H), 6.49 (s, 1 H), 6.05 (s, 1 H), 3.88 (s, 6 H), 2.90–2.72 (m, 3 H), 2.49–2.32 (m, 2 H), 2.39 (s, 3 H). Anal. ($C_{13}H_{16}O_2S$) C, H, S.

6,7-Dimethoxy-2-(methylthio)naphthalene (17). To a suspension of DDQ (227 mg, 1 mmol) in CH_2Cl_2 (5 mL) was added dropwise a solution of dihydronaphthalene (200 mg, 0.85 mmol) in CH_2Cl_2 (2 mL). The resulting mixture was stirred for 10 min at room temperature, followed by the addition of *n*-hexane (5 mL) and filtration. Chromatography with petroleum ether–ethyl acetate (9:1) on silica gel afforded 192 mg (97%) of naphthalene **17**: mp 92–94 °C (from CH_2Cl_2–pentane); NMR ($CDCl_3$) δ 7.59 (d, 1 H, J = 8.5 Hz), 7.53 (d, 1 H, J = 1.9 Hz), 7.25 (dd, 1 H, J = 8.5 and 1.90 Hz), 7.07 (s, 1 H), 7.03 (s, 1 H), 3.99 (s, 6 H), 2.56 (s, 3 H).

6,7-Dimethoxy-2-(methylthio)tetralin (18). Et_3SiH (2.0 g, 17.2 mmol) was added to a solution of dihydronaphthalene **16** (1.0 g, 4.2 mmol) in anhydrous benzene (20 mL) at room temperature. To the resulting mixture, after stirring for 10 min, was added dropwise CF_3CO_2H (4 g) over 10 min with stirring and cooling in an ice bath, followed by stirring for 5 h at room temperature. The reaction mixture was evaporated to give an oily residue, which was dissolved in ether (20 mL), washed with two portions of saturated $NaHCO_3$ (60 mL), dried on anhydrous Na_2CO_3, and finally purified on a silica gel column with 5% ethyl acetate in petroleum ether to yield 940 mg (93%) of tetralin **18**: mp 48–50 °C; recrystallization from ether–pentane gave mp 49–50 °C; NMR ($CDCl_3$ δ 6.57 (s, 1 H), 6.55 (s, 1 H), 3.83 (s, 6 H), 3.2–2.6 (m, 5 H), 2.3–2.0 (m, 2 H), 2.17 (s, 3 H). Anal. ($C_{13}H_{18}O_2S$) C, H, S.

(6,7-Dihydroxy-2-naphthalenyl)dimethylsulfonium Bromide (7). To a solution of **17** (2.5 g) in CH_2Cl_2 (10 mL) was added dropwise BBr_3 (23 mL, 1 M solution in CH_2Cl_2) with cooling in a dry ice–acetone bath under nitrogen. The mixture was warmed gradually to room temperature by stirring for an additional 10 h at room temperature. To the resulting mixture was added dry MeOH (13 mL) with cooling in an ice bath. The

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mixture was stirred for 5 h at room temperature, and the solvent was removed in vacuo to give a solid, which was washed with a mixture of ethyl acetate and ether (1:1) to afford 1.95 g (88.6%) of **7**: mp 150–151 °C; recrystallization from MeOH/ethyl acetate gave mp 151 °C; NMR (Me_2SO) δ 8.34 (d, 1 H, J = 1.9 and 8.9 Hz), 7.30 (s, 1 H), 7.26 (s, 1 H), 3.30 (s, 3 H). Anal. ($C_{11}H_{10}O_2S$) C, H, S.

(6,7-Dihydroxy-2-tetralinyl)dimethylsulfonium Bromide (6). A solution of BBr_3 (1.0 mL, 1 M solution in CH_2Cl_2) was added to a solution of **18** (180 mg) in CH_2Cl_2 (5 mL) cooled in a dry ice–acetone bath under nitrogen. After stirring for 30 min, the reaction mixture was warmed to room temperature, and then stirring was continued for 15 h at room temperature. Anhydrous MeOH (2 mL) was added to the resulting mixture with cooling in a dry ice bath, followed by stirring for 3 h at room temperature. Removing the solvent gave a residue, which solidified. The residue was washed with ethyl acetate to yield 218 mg of white solids (dec pt 125–127 °C). Recrystallization from MeOH–ethyl acetate afforded 166 mg (72%) of colorless crystals: dec pt 126–127 °C; NMR (D_2O) δ 6.70 (s, 2 H), 3.85–3.65 (m, 1 H), 3.30–2.60 (m, 4 H), 2.95 (s, 3 H), 2.93 (s, 3 H), 2.50–1.85 (m, 2 H). Anal. ($C_{12}H_{17}SO_2Br$) C, H, S.

Preparation of Brain Slices. Male Swiss Webster mice (Harlan labs) were used for all experiments. After the mice were decapitated, the brains were removed, and the striatal tissue rostral to the anterior commissures were dissected.[20] The tissue was cut into 0.5 × 0.5 mm sections with a McIlwain tissue chopper 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), glucose (11). It was bubbled with a 95% O_2–5% CO_2 mixture and adjusted to pH 7.2 with NaOH. The slices were incubated for 20 min with [^{3}H]choline at a final concentration of 0.1 M. This low concentration of [^{3}H]choline favors the selective uptake of choline into cholinergic neurons through a high-affinity uptake system.[21] After the slices were rinsed, they were transferred to a superfusion system[22] and superfused with medium at 37 °C at a constant rate of 0.5 m/min. Fractions were collected every 5 min, starting at 40 min after the onset of superfusion. Transmitter release was induced by superfusion with medium containing 12.5 M K^+ for 5 min starting at 60 (S_1) and 90 (S_2) min after the onset of the superfusion. Drugs were added to the medium 15 min before S_2. The slices were superfused for a total of 110 min. 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). This was calculated by correcting the tissue content of each fraction for the radioactivity lost to the medium. The K^+-induced increase in release (denoted S_2 and S_1) is the sum of the fractional release obtained following the addition of the high K^+ medium above the baseline of spontaneous outflow (Sp). The latter is the fractional release of tritium obtained during the 5-min interval prior to the addition of the high K^+ medium. The effects of dopaminergic agonists on K^+-induced release were evaluated by determining the ratio S_2/S_1 for control- and drug-treated slices. The effect of drugs on the spontaneous release of tritium was evaluated by determining the fractional release of radioactivity during the 5-min intervals preceding S_2 and S_1, respectively, and was expressed as the ratio (Sp_2/Sp_1).

The tritium released by the high K^+ medium was not chemically characterized in these experiments, since several previous studies have demonstrated that radioactive acetylcholine formed from radiolabeled choline can be released from brain slices by K^+-depolarization.[23-28] 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 has been shown to inhibit the depolarization-induced release of acetylcholine by the process of feedback inhibition.[29,30] In the present study, physostigmine was omitted from the medium. Under our conditions, the K^+-induced release of tritium is completely dependent on the presence of calcium ions in the superfusion medium.

Registry No. **5**, 89017-40-3; **6**, 89017-41-4; **7**, 89017-42-5; **8**, 56221-32-0; (*R*)-**9**, 89017-43-6; (*S*)-**9**, 89017-44-7; **10**, 89017-45-8; **11**, 5333-34-6; **12**, 13575-74-1; **13**, 13575-75-2; **15**, 35491-96-4; **16**, 89017-46-9; **17**, 33212-93-0; **18**, 89017-47-0; Ach, 51-84-3; veratrol, 91-16-7; tetralol, 529-33-9; succinic anhydride, 108-30-5.

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Potential Inhibitors of Nucleotide Biosynthesis. 2. Halomethyl Ketone Derivatives of Pyrimidine Nucleosides[1]

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Several halomethyl ketone derivatives of pyrimidine nucleosides have been prepared for evaluation as cytotoxic agents. The first series are 1-(8-halo-2,5,6,8-tetradeoxy-β-D-*erythro*-oct-7-ulofuranosyl)thymines (**7–9**), whereas the second type are halo derivatives of acetophenone (**12–14** and **16**). These compounds are cytotoxic, and one (**13**) showed activity against the P388 leukemia in vivo.

A detailed rationale for the preparation of nucleosides containing a chemically reactive function attached through a spacer to C-5′ that may act as active-site-directed, irreversible inhibitors of enzymes that metabolize the corresponding nucleotides has been presented.[3] As a part of this program, we prepared diazo ketone derivatives of thymidine and uridine,[4] since such a functional group should react under in vivo conditions with protonated guanidine groups that act as binding points for the phosphate moieties for the corresponding nucleotides.[5] An

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0022-2623/84/1827-0680$01.50/0