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# EFFECT OF A SULFONIUM ANALOG OF DOPAMINE ON THE DEPOLARIZATION-INDUCED RELEASE OF [$^3$H]ACETYLCHOLINE FROM MOUSE STRIATAL SLICES

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**Abstract**—We have synthesized a chemical analog or dopamine in which the amino group has been replaced by a charged dimethylsulfonium group. The dopaminergic activity of this drug was evaluated by determining its ability to inhibit the depolarization-evoked release of [$^3$H]acetylcholine from mouse striatal slices. The slices were preincubated with [$^3$H]choline (0.1 $\mu$M) and then superfused in physiological medium. [$^3$H]Acetylcholine release was induced by exposure of the slices to a high potassium medium (12.5 mM) for 5 min. The sulfonium analog of dopamine, dopamine, and apomorphine inhibited the evoked [$^3$H]acetylcholine release with $IC_{50}$ values of approximately 10, 2.0, and 0.3 $\mu$M respectively. The inhibition by the sulfonium analog was reversed by fluphenazine (1 $\mu$M), suggesting that the inhibition of [$^3$H]acetylcholine release was due to the activation of dopaminergic receptors. The sulfonium analog also inhibited the uptake of [$^3$H]dopamine into striatal slices and caused the release of exogenously taken up [$^3$H]dopamine from these slices. The release of [$^3$H]dopamine by the sulfonium analog was inhibited by cocaine (3 $\mu$M), suggesting that the drug-induced release of [$^3$H]dopamine was dependent on the carrier-mediated uptake of the sulfonium analog into dopaminergic neurons. The inhibition of the evoked [$^3$H]acetylcholine release by high concentrations (30 and 60 $\mu$M) of the sulfonium analog did not appear to be mediated by endogenous dopamine release, since the analog still inhibited [$^3$H]acetylcholine release from slices after reserpine-$\alpha$-methyl-*p*-tyrosine treatment. However, the inhibitory effect of the sulfonium analog at 10 $\mu$M was reduced by reserpine-$\alpha$-methyl-*p*-tyrosine treatment, suggesting that the inhibition at lower concentrations was mediated through endogenous DA release. These results suggest that a charged compound can act as a substrate for the dopamine carrier and can activate the dopamine receptor regulating acetylcholine release. They also indicate that the nitrogen on the dopamine molecule is not essential for dopamine agonist activity.

In recent years, there has been a large amount of research on the chemistry, physiology, and pharmacology of dopaminergic neurotransmission in the central nervous system [1–5]. An abnormality of dopaminergic neurotransmission has been implicated in several diseases including Parkinson's disease, schizophrenia, Huntington's chorea, tardive dyskinesia, certain cardiovascular disorders, obesity and neuroendocrine disorders associated with the control of prolactin secretion[5]. At present, there is considerable interest in the development of potent and selective dopamine (DA) receptor agonists for use in the treatment of these diseases or in the development of animal models of disease states.

The relationship between the molecular structure of DA and DA agonist activity has been the subject of several investigations. Studies have been directed toward determining the molecular conformations and configurations that are required to produce DA receptor activity [6–9]. In addition, the effects of substitution on the side chain nitrogen have also been studied [7]. However, there is little information available on whether the nitrogen itself is necessary for dopaminergic receptor acitivity.

We have synthesized an analog of DA in which the amino group has been replaced by a sulfonium group [10]. In our initial studies, we found that this compound (Fig. 1) was able to displace [$^3$H]DA from a crude striatal membrane fraction, and the concentration that inhibited the specific binding of [$^3$H]DA by 50% was approximately one-tenth that of DA. However, the maximum inhibition of [$^3$H]-DA binding by the sulfonium analog was significantly less than that of DA. In addition, questions have been raised about the significance of the [$^3$H]DA binding site [5, 11]. Therefore, to evaluate the dopaminergic activity of the sulfonium analog of DA, we determined its effect on the potassium ($K^+$)-induced release of [$^3$H]acetylcholine from striatal slices. In this functional system, the activation of DA receptors has been shown to inhibit the $K^+$-induced release of [$^3$H]acetylcholine [12–15]. We report that the sulfonium analog of DA can inhibit [$^3$H]acetylcholine release by both directly activating DA receptors as well by releasing endogenous DA.

## MATERIALS AND METHODS

*Preparation of brain slices.* Male Swiss–Webster mice (Harlan Sprague–Dawley Indianapolis, IN) were used for all experiments. After decapitation,

* Deceased.

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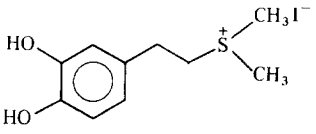


Fig. 1. Sulfonium analog of DA: 2-(3,4-dihydroxyphenyl) ethyl dimethylsulfonium iodide.

the brains were removed, and the striatal tissue rostral to the anterior commissures was dissected [16]. The tissue was cut into 0.5 mm × 0.5 mm sections using 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; 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 with [$^3$H]choline at a final concentration of 0.1 $\mu$M. This low concentration of [$^3$H]choline favors the selective uptake of choline into cholinergic neurons through a high affinity uptake system [17]. After rinsing, the slices were transferred to a superfusion system [18] and superfused with medium at 37° at a constant rate of 0.5 ml/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 mM $K^+$ 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 hav demonstrated that radioactive acetylcholine formed from radiolabeled choline can be released from brain slices by $K^+$-depolarization [19–24]. 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 [24, 25]. In the present study, physostigmine was omitted from the medium. Under our conditions, the $K^+$-induced release of tritium was completely dependent on the presence of calcium ions in the superfusion medium (data not shown).

*Measurement of the uptake and release of* [$^3$H]*DA.* For the uptake studies, the striatal tissue was cut at 90° angles using the McIlwain chopper (Brinkmann Instruments, Westbury, NY) to a thickness of 0.2 mm by 0.2 mm. The slices were dispersed in Krebs–Ringer Hepes (*N*-2 hydroxyethylpiperazine-*N*$^1$-ethanesulfonic acid) buffered medium [26] containing pargyline (0.1 mM). After centrifugation at 500 *g* for 2 min, the supernatant fraction was discarded, and the slices were resuspended in a volume of medium such that 0.2-ml aliquots of this suspension would be equivalent to 10 mg of tissue. The aliquots were added to flasks and preincubated for 5 min at 37°. [$^3$H]DA was then added at a final concentration of 0.04 $\mu$m, and the incubation was continued for an additional 5 min. The slices were separated from the medium by filtration on Whatman no. 1 filter paper and washed with 10 ml of cold medium. The filter paper discs with the slices were placed in a counting vial with 1 ml of water for 30 min. Scintillation fluid was added, and the radioactivity was determined by liquid scintillation counting. All values for [$^3$H]DA uptake determined at 37° were corrected for the uptake of [$^3$H]DA at 0°. Based on a study in which the amount of [$^3$H]DA in the water extract was determined by Dowex 50 chromatography (Uretsky and Snodgrass, unpublished results), [$^3$H]DA accounted for more than 90% of the radioactivity in the slices.

The release of [$^3$H]DA previously taken up into striatal slices was determined as previously described [27–29]. In this procedure, the slices were incubated with [$^3$H]DA (0.05 to 0.1 $\mu$M) for 20 min, washed extensively, and then incubated with the drug for 15 min. The effect of the drug on the amount of [$^3$H]-DA in the incubation medium and the tissue was determined. [$^3$H]DA in tissue and medium was separated from deaminated metabolites by Dowex 50 chromatography, and the radioactivity in the appropriate eluates from the Dowex columns was determined by liquid scintillation chromatography [28]. Release is expressed as [$^3$H]DA in the medium as a percentage of the [$^3$H]DA in tissue + medium [27–29].

*Statistics.* Statistical comparisons were performed using the two-tailed Mann Whitney U test [30].

*Materials.* The sulfonium analog of DA was synthesized as previously described [10]. [*Methyl*-$^3$H]-Choline chloride (15 Ci/mmole) was purchased from Amersham International Ltd. (Amersham, U.K.). Dopamine [7-$^3$H(N)] (30.4 Ci/mmole) was purchased from the New England Nuclear Co. (Boston, MA). Dopamine (3-hydroxytyramine), apomorphine, and pargyline HCl were purchased from the Sigma Chemical Co. (St. Louis, MO).

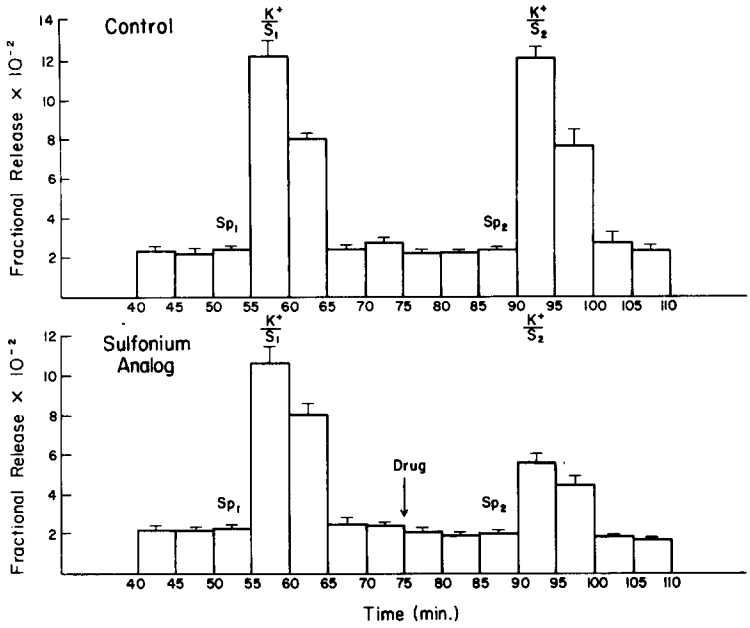


Fig. 2. Effect of the sulfonium analog of DA on the $K^+$-induced release of [$^3$H]acetylcholine from mouse striatal slices. $Sp_1$ and $Sp_2$ represent the spontaneous release of tritium during the 5-min intervals preceding the addition of the high $K^+$ medium. $S_1$ and $S_2$ represent the $K^+$-evoked stimulation of tritium release. The drug (sulfonium analog) was added at the arrow. Each value is the mean ± S.E.M. of seven determinations.

## RESULTS

*Inhibition of potassium-induced* [$^3$H]*acetylcholine release produced by the sulfonium analog of DA, DA, and apomorphine.* The effect of the sulfonium analog (30 μM) on the $K^+$-induced release of [$^3$H]-acetylcholine is summarized in Fig. 2. After the addition of the sulfonium analog to the medium, there was approximately a 46% reduction in the $K^+$-induced release of [$^3$H]acetylcholine.

As shown in Fig. 3, apomorphine and DA also inhibited the $K^+$-induced release of [$^3$H]acetylcholine from striatal slices in a concentration-dependent manner. In agreement with a previous report [13], apomorphine was found to be approximately one hundred times more potent than DA in this system. The sulfonium analog of DA inhibited the $K^+$-induced release of [$^3$H]acetylcholine in a concentration-dependent manner and was about five times less potent than DA.

*Effect of fluphenazine on the inhibition of the* $K^+$*-induced* [$^3$H]*acetylcholine release produced by the sulfonium analog of DA and apomorphine.* As shown in Table 1, the sulfonium analog (30 μM) and apomorphine (1.0 μM) produced a 57 and 48% decrease in the release of [$^3$H]acetylcholine ($S_2/S_1$) induced by high $K^+$. However, fluphenazine significantly reversed the inhibitory effect produced by both the sulfonium analog and apomorphine on the release of [$^3$H]acetylcholine evoked by $K^+$. None of the drugs tested significantly altered the spontaneous release of tritium ($Sp_2/Sp_1$).

*Effect of the sulfonium analog of DA on the uptake and release of* [$^3$H]*DA.* To determine whether the sulfonium analog of DA could alter dopaminergic mechanisms in the slices, we studied the effect of these drugs on [$^3$H]DA uptake and release. As shown in Fig. 4A, the sulfonium analog inhibited the uptake of [$^3$H]DA into striatal slices with an $IC_{50}$ of approximately 30 μM. The analog also increased the percentage of exogenously taken up [$^3$H]DA released from striatal slices ((Fig. 4B). For these studies, total [$^3$H]DA represented an average of 46.7 ± 12.1% [15]

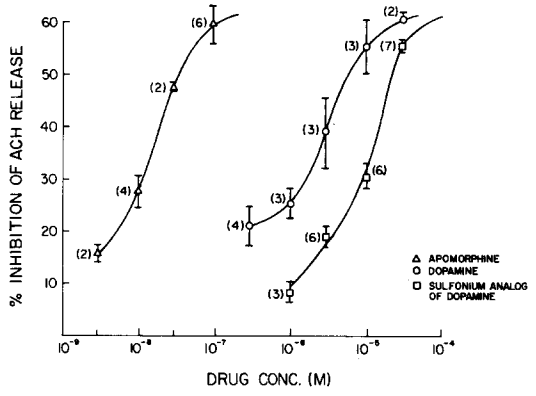


Fig. 3. Effect of apomorphine, DA, and the sulfonium analog of DA on the $K^+$-evoked [$^3$H]acetylcholine release from striatal slices. Striatal slices were incubated with [$^3$H]-choline and then superfused for 110 min. [$^3$H]Acetylcholine release was evoked by two successive exposures ($S_1$ and $S_2$) to $K^+$ at 60 and 90 min. The drugs were added 15 min before $S_2$. The results are expressed as the percentage inhibition of the control (no drugs) $S_2/S_1$ ratio produced by the drugs. The control $S_2/S_1$ ratio was 0.96 ± 0.018 (N = 33). Each value represents the mean and either the S.E.M. or range (in the case of two observations). The number of determinations is given in the parentheses.

Table 1. Effect of fluphenazine on the inhibition of $K^+$-evoked [$^3$H]acetylcholine release produced by the sulfonium analog of DA and apomorphine*

| Treatment | N | Fractional Tritium Release $S_2/S_1$ | $Sp_2/Sp_1$ |
|---|---|---|---|
| Control | 7 | 1.00 ± 0.05 | 0.98 ± 0.05 |
| Apomorphine (1 μM) | 6 | 0.52 ± 0.02† | 0.98 ± 0.35 |
| Sulfonium analog (30 μM) | 5 | 0.43 ± 0.03† | 0.88 ± 0.07 |
| Fluphenazine (1 μM) | 4 | 0.81 ± 0.03 | 0.89 ± 0.05 |
| Fluphenazine + sulfonium analog | 6 | 0.80 ± 0.05‡ | 1.04 ± 0.07 |
| Fluphenazine + apomorphine (1 μM) | 6 | 0.76 ± 0.03§ | 0.85 ± 0.04 |

* $S_2/S_1$ refers to the ratio of the $K^+$-induced increase in tritium release for two periods of stimulation. $Sp_2/Sp_1$ refers to the ratio of the spontaneous outflow of tritium during the 5-min periods preceding the first and second period of stimulation. The sulfonium analog and apomorphine were added 15 min before $S_2$. Fluphenazine (1 μM), when present, was added at the start of the superfusion. Each value is the mean S.E.M.

† $P < 0.05$, when compared to control.

‡ $P < 0.05$, when compared to the sulfonium analog alone.

§ $P < 0.05$, when compared to apomorphine alone.

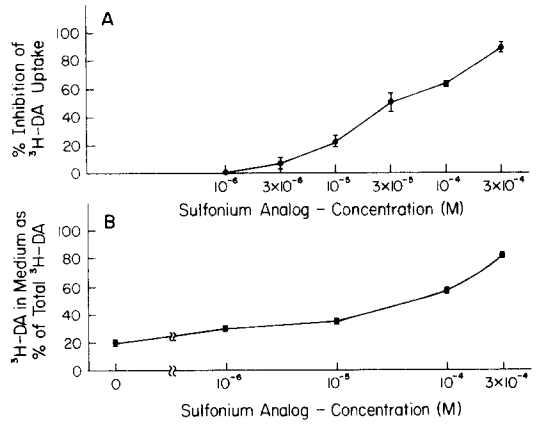


Fig. 4. Effect of the sulfonium analog on the uptake and release of [$^3$H]DA. (A) Uptake: Striatal slices were preincubated for 10 min in medium containing different concentrations of the sulfonium analog. [$^3$H]DA (0.04 μM) was then added, and the incubation was continued for an additional 5 min. The slices were then separated from the medium by filtration, and the amount of radioactivity in the slices was determined. The net uptake of [$^3$H]DA was determined by subtracting blank values corresponding to the accumulation of [$^3$H]DA into slices at 0°. Results are the mean ± S.E.M. of three to five determinations. The accumulation of [$^3$H]DA in control slices corresponded to a T/M ratio of 34.8 ± 3.9 (N = 5). (B) Release: Striatal slices were incubated with [$^3$H]DA (0.05–0.1 μM) for 15 min. After repeated washing, they were then placed in a medium containing various quantities of the sulfonium analog for 15 min. [$^3$H]DA was measured in tissue and medium as previously described (see Materials and Methods). The total [$^3$H]DA was 23,888 ± 1,355 dpm (N = 29). Each value is the mean ± S.E.M. of three to eleven determinations.

of total radioactivity, and the various concentrations of analog did not significantly change this value from that of controls (data not shown). The addition of cocaine (3 μM), an inhibitor of DA uptake, to the medium had little effect on the control release of [$^3$H]DA but significantly inhibited the release of [$^3$H]DA produced by the sulfonium analog (Fig. 5). These studies, which demonstrate that the sulfonium analog can release [$^3$H]DA, suggest that the inhibition of the $K^+$-induced [$^3$H]acetylcholine release produced by the sulfonium analog could be mediated through the release of endogenous DA.

*Effect of reserpine-α-methyl-*p*-tyrosine treatment on the inhibition of $K^+$-induced [$^3$H]acetylcholine release produced by amphetamine, apomorphine and the sulfonium analog of DA.* As shown in Table 2, amphetamine, apomorphine and the sulfonium analog significantly inhibited the $K^+$-induced release of [$^3$H]acetylcholine from striatal slices of control animals as reflected in the decreased $S_2/S_1$ ratio. Striatal slices were also prepared from mice pretreated with reserpine (5 mg/kg, i.p., 20 hr previously) and α-methyl-*p*-tyrosine (200 mg/kg, i.p., 1.5 hr previously) and then superfused in medium containing α-methyl-*p*-tyrosine (0.25 mM). Under these conditions, amphetamine no longer produced an inhibition of the $K^+$-induced release of [$^3$H]acetylcholine, while apomorphine remained an effective inhibitor (Table 2). The sulfonium analog acted more like apomorphine after reserpine and α-methyl-*p*-tyrosine treatment. Although its inhibitory effect was reduced at a concentration of 10 μM, the sulfonium analog produced a significant inhibition of $K^+$-induced [$^3$H]acetylcholine release at 30 and 60 μM, despite the pretreatment with the DA-depleting drugs.

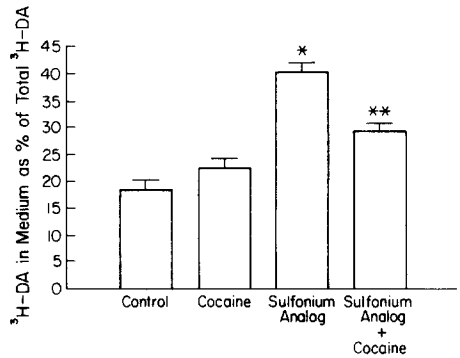


Fig. 5. Effect of cocaine on the release of [$^3$H]DA produced by the sulfonium analog. Striatal slices were incubated with [$^3$H]DA (0.05 μM) for 15 min, repeatedly washed, and then incubated in control medium or medium containing drugs for 15 min. [$^3$H]DA was measured in tissue and medium. The results are expressed as [$^3$H]DA in the medium as a percentage of total [$^3$H]DA in tissue and medium. The total [$^3$H] DA was 30,523 ± 2,003 dpm (N = 28). The percentage of total counts present as [$^3$H]DA in the control, sulfonium analog, cocaine, and cocaine and sulfonium analog groups was 29.2 ± 3.1% (3), 29.7 ± 4.2% (3), 38.8 ± 5.1 (4), and 37.8 ± 2.0% (4) respectively (these values were not statistically different). Each value is the mean ± S.E.M. six to eight determinations. Key: (*) $P < 0.05$, compared to control; and (**) $P < 0.05$, compared to the sulfonium analog group.

Table 2. Effect of treatment with reserpine and α-methyl-*p*-tyrosine on the inhibition of $K^+$-induced [$^3$H]-acetylcholine release produced by amphetamine, apomorphine, and the sulfonium analog of DA*

| | Concn (μM) | Fractional tritium release | | | |
|---|---|---|---|---|---|
| | | $S_2/S_1$ (control) | % Zero concn | $S_2/S_1$ (reserpine + α-methyl-*p*-tyrosine | % Zero concn |
| Sulfonium analog | 0 | 0.92 ± 0.03 (5) | 100 ± 3 | 0.99 ± 0.03 (8) | 100 ± 3 |
| | 10 | 0.65 ± 0.04† (8) | 71 ± 4 | 0.91 ± 0.05 (13) | 92 ± 5 |
| | 30 | 0.46 ± 0.01† (7) | 50 ± 1 | 0.60 ± 0.03† (14) | 61 ± 3 |
| | 60 | 0.46 ± 0.03† (3) | 50 ± 3 | 0.39 ± 0.03† (4) | 40 ± 3 |
| Amphetamine | 0 | 0.91 ± 0.02 (11) | 100 ± 2 | 0.98 ± 0.03 (9) | 100 ± 3 |
| | 0.1 | 0.75 ± 0.06† (3) | 82 ± 7 | 0.97 ± 0.10 (3) | 99 ± 10 |
| | 1.0 | 0.32 ± 0.03† (4) | 36 ± 4 | 0.90 ± 0.04 (6) | 92 ± 4 |
| | 10.0 | 0.33 ± 0.02† (5) | 37 ± 2 | 0.96 ± 0.03 (7) | 98 ± 3 |
| | 30.0 | | | 0.94 ± 0.12 (7) | 96 ± 12 |
| Apomorphine | 0 | 0.92 ± 0.02 (6) | 100 ± 2 | 1.07 ± 0.06 (5) | 100 ± 6 |
| | 0.03 | 0.66 ± 0.05 (7) | 72 ± 6 | 0.68 ± 0.04† (3) | 63 ± 4 |
| | 0.10 | 0.37 ± 0.02† (6) | 40 ± 3 | 0.48 ± 0.03† (7) | 44 ± 3 |

* Each value is the mean ± S.E.M. with the number of determinations given in parentheses.
† *$P < 0.05$, compared to the respective non-drug-treated controls (0 μM), using the Mann Whitney U test.

## DISCUSSION

To determine whether the sulfonium analog of DA possessed DA agonist activity, we have studied the effect of this compound on the depolarization-evoked release of [$^3$H]acetylcholine from striatal slices. Although the sulfonium analog of DA has been shown previously to inhibit [$^3$H]DA binding to a crude rat striatal membrane preparation [10], the functional role of this binding site, which has been classified as a $D_3$ site [5, 11], is unclear. In contrast, in the striatal slice model used in the present study, DA receptor activation affected the function of a specific type of neuron, i.e. the cholinergic neuron. The DA receptor regulating [$^3$H]acetylcholine release appears to be a $D_2$ receptor [13, 31, 32], which has been correlated with the functional effects produced by dopaminergic agonists [5]. Striatal acetylcholine neurons are involved in extrapyramidal motor function and may mediate the changes in motor behavior produced by DA receptor activation [33–36]. Consequently, it is likely that drugs that activate dopaminergic receptors regulating [$^3$H]-acetylcholine release would be capable of producing functional changes in motor behavior.

The present studies show that the sulfonium analog of DA can inhibit the $K^+$-induced release of [$^3$H]-acetylcholine from striatal slices. Thus, this compound exerts an effect similar to that of DA and dopaminergic agonists. The potency of the sulfonium analog was approximately 1/1000 that of apomorphine and 1/5 that of DA. Fluphenazine, a DA receptor antagonist which reversed the inhibitory effect of apomorphine on the evoked release of [$^3$H]-acetylcholine, reversed the inhibition produced by the sulfonium analog of DA (Table 1). These studies suggest that the sulfonium analog of DA is able to produce the activation of the DA receptor that regulates the $K^+$-induced release of [$^3$H] acetylcholine from striatal slices.

The inhibition of the $K^+$-induced [$^3$H]acetylcholine release by DA agonists can occur either by a direct action on the DA receptor site or by an indirect action by blocking DA reuptake or releasing DA from the dopaminergic nerve terminal [15]. Although it is a permanently charged molecule, the sulfonium analog was able to both inhibit [$^3$H]DA uptake into striatal slices and release exogenously taken up [$^3$H]DA at concentrations that also inhibited the evoked release of [$^3$H]acetylcholine (Fig. 4, A and B). Therefore, the possibility exists that the inhibition of evoked [$^3$H]acetylcholine release produced by the sulfonium analog is mediated by an enhancement in the synaptic concentration of DA.

To test this hypothesis, the effect of the sulfonium analog on the evoked release of [$^3$H]acetylcholine was studied in striatal slices under conditions in which DA synthesis was inhibited by α-methyl-*p*-tyrosine and DA storage sites were depleted by reserpine. Under these conditions, amphetamine, which normally is able to inhibit the evoked release of [$^3$H]acetylcholine, did not produce a significant decrease in the evoked release of [$^3$H]acetylcholine (Table 2). In contrast, apomorphine, which acts directly on dopamine receptors, inhibited the evoked [$^3$H]acetylcholine release from both control and reserpine-α-methyl-*p*-tyrosine-treated slices (Table 2). The sulfonium analog at concentrations of 30 and 60 μM produced a similar degree of inhibition of [$^3$H]-acetylcholine release in treated and control slices, although it had less of an inhibitory effect in the treated slices when the concentration in the medium was 10 μM. These results show that the sulfonium analog of DA can produce an effect similar to that of apomorphine, indicating that the inhibition by high concentrations of the sulfonium analog is caused by a direct activation of the DA receptors regulating acetylcholine release.

DA is present in physiological solution as both a charged and uncharged molecule [7]. At present it is not clear which form of the molecule interacts with

the recognition site of the receptor or the DA uptake system. The observation that the sulfonium analog of DA, a permanently charged molecule, can block [$^3$H]DA uptake (Fig. 4A) suggests that a charged molecule can interact with the DA carrier. The sulfonium analog was also able to cause the release of [$^3$H]DA from striatal slices, and this release was inhibited by cocaine (Fig. 5), which has been shown to inhibit the uptake of [$^3$H]DA into dopaminergic nerve terminals [37]. This observation suggests that the charged sulfonium analog is a substrate for the DA carrier and is transported into DA nerve terminals where it can cause the release of DA probably from storage sites. The conclusion that a charged compound is a substrate for a catecholamine carrier is in agreement with the results of studies on the norepinephrine (NE) carrier, in which the p$K_a$ of various compounds was correlated with their ability to inhibit [$^3$H]NE uptake into a variety of sympathetically innervated tissues [38, 39]. The results of several previous studies have suggested that DA and DA receptor agonists and antagonists interact with DA receptors in the uncharged form [9, 39–42]. These conclusions, however, were mainly based on the effects of agonist and antagonist drugs which were primary, secondary, and tertiary amines. Thus, these studies are not definitive because both charged and uncharged forms of the drugs would be expected to be present in solution [7]. In addition, the proposal that a DA receptor antagonist, butaclamol, binds to the DA receptors in the uncharged form [41, 42] may not apply to DA receptor agonist drugs, since DA receptor agonists and antagonists may bind to different receptor recognition sites [1, 5, 11]. In the present study, the sulfonium analog produced a response characteristic of a DA receptor agonist, suggesting that a charged molecule can effectively interact with the DA receptor.

The sulfonium analog molecule contains a sulfur atom instead of a nitrogen atom on the phenethylamine side chain. The pharmacological effects of this compound suggest that the side chain nitrogen of DA is not essential for either DA uptake or DA agonist activity.

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