shown in Table 1. Similarly, total cholesterol content in serum of rats administered aspirin or salicylic acid was decreased to 79–92% of that of control rats. We have also found that the amounts of salicylic acid in the sera of rats administered aspirin and salicylic acid were 0.46 and 0.64 μmole/ml of serum respectively.

The elevation of the acyltransferase activity in serum of rats administered aspirin or salicylic acid orally seems to be due mainly to the decrease of serum free cholesterol content. In addition, the decrease of cholesterol content in rat serum by the administration of aspirin or salicylic acid may be due to the increase of excretion of cholesterol into bile, the increase of enzymatic conversion of cholesterol to bile acid, and/or the decrease of absorption of cholesterol from intestine. Experiments to clarify these problems are in progress now.

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Similar potency ratios of amphetamine optical isomers for inhibition of dopamine uptake by synaptosomes of corpus striatum, olfactory tubercle and prefrontal cortex of the rat

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Although comparable doses of the optical isomers of amphetamine (AMPH) have been shown to produce or exacerbate psychotic symptoms in man [1–3], it has been shown in electrophysiological [4] and biochemical [5, 6] experiments that D-AMPH is much more potent than L-AMPH in its actions on dopamine (DA) neurons projecting to the corpus striatum from the substantia nigra (SN). The near equipotence of the isomers in their psychotogenic effects is thus difficult to interpret in the framework of the dopamine hypothesis of schizophrenia. However, recent evidence suggests that DA neurons of the ventral tegmental area (VTA) rather than those of the SN may be involved in schizophrenia [7–9]. In humans and in rats, VTA DA neurons innervate several limbic and cortical structures including nucleus accumbens, olfactory tubercle, prefrontal, cingulate and entorhinal cortex [10, 11]. Mesocortical DA neurons in particular are a unique subpopulation of DA neurons which appear to lack DA autoreceptors [12, 13]. Thus, mesocortical DA neurons, in contrast to mesolimbic and SN DA neurons, do not show decreases in rate of impulse flow or in tyrosine hydroxylase activity to low doses of the DA receptor agonist apomorphine. It is therefore possible that mesocortical DA neurons may have a unique response to other dopaminergic drugs, including the optical isomers of amphetamine. To test this possibility, we have established a method of selectively measuring DA uptake by DA nerve terminals and have tested the relative potencies of D- and L-AMPH on mesolimbic and mesocortical terminals as compared to nigrostriatal terminals. We have found that there is no difference in the potency ratios for the AMPH isomers in the three areas. D-AMPH is about 5-fold more potent than L-AMPH in each terminal region. A preliminary report of these findings has been published [14].

Materials and methods

Female Holtzman albino rats weighing 200–225 g were used in all experiments. Tissue preparation and uptake assays were done by a modified method of Horn *et al.* [15]. Briefly, rats were killed with chloroform asphyxiation, and brains were quickly removed and chilled in ice-cold saline. Samples of frontal cortex were taken as described by Bockaert *et al.* [16]. These consisted of a medial-dorsal wedge of cortex rostral to the nucleus accumbens. Samples of corpus striatum and olfactory tubercle were dissected bilaterally. Tissues were weighed and then homogenized with a Teflon pestle in glass tubes in 20 vol. of 250 mM sucrose. The homogenate was centrifuged at 1000 *g* for 10 min, and the supernatant fraction was used for incubations. Aliquots (100 μl) of the supernatant fraction were incubated with 1.9 ml of a modified Krebs–Henseleit buffer (95 mM NaCl, 4.7 mM KCl, 1.25 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 4.9 mM pyruvate, 5.4 mM fumarate, 4.9 mM L-glutamate, 11.5 mM glucose, 12.5 μM nialamide, 0.2 mg/ml sodium EDTA, 0.2 mg/ml ascorbic acid) in a shaking water bath (37°) under a 5% CO_2/95% O_2 atmosphere. Drugs were added 5 min before [^{3}H]-DA (10^{-7} M). Incubations were terminated after 5 additional min by rapid filtration through 2.1 cm Whatman GF/C filters and washed twice with 5 ml of ice-cold buffer. Preliminary experiments showed linear accumulation of [^{3}H]-DA for at least 5 min in all three brain area preparations. Filters were extracted with toluene-base scintillation mixture for at least 30 min followed by estimation of tritium with a Beckman scintillation counter.

[^{3}H]-Dopamine (21.5 Ci/mmole) was obtained from New England Nuclear, AMPH isomers from Smith, Kline & French Laboratories, fluoxetine from Lilly Research Lab-

oratories, desipramine from USV Laboratories, and amfonelic acid from Sterling Winthrop Research Institute.

Results and discussion

Contrary to the striatum and olfactory tubercle, the dopaminergic innervation of prefrontal cortex is relatively sparse, compared to its noradrenergic and serotonergic innervations [10, 11, 17–21]. To test whether uptake of [^{3}H]-DA by norepinephrine (NE) and serotonin (5-HT) terminals would contribute significantly to total uptake in synaptosomal preparations, a relatively selective NE re-uptake blocker, desipramine (DMI), and a selective 5-HT re-uptake blocker, fluoxetine, were tested for inhibition of [^{3}H]-DA accumulation. DMI and fluoxetine at 10^{-6} M had little or no effect on [^{3}H]-DA accumulation in striatal or olfactory tubercle synaptosomes. In the prefrontal cortex, however, DMI and fluoxetine inhibited uptake by 67 and 24% respectively (Fig. 1). Uptake inhibition by DMI plus fluoxetine was not different from DMI alone (Dunnett's *t*-test). These data suggest substantial uptake of [^{3}H]-DA by NE and/or 5-HT neuron terminals in prefrontal cortex but not in olfactory tubercle or striatum.

Residual accumulation of [^{3}H]-DA in the presence of DMI and fluoxetine was inhibited by a potent DA uptake blocker, amfonelic acid (AFA) [22, 23]. AFA at 10^{-6} M maximally inhibited residual [^{3}H]-DA accumulation in all three DA terminal regions (75% inhibition), and at 2×10^{-8} M it produced about 50% inhibition in all areas. These findings indicated that DA uptake in the presence of DMI and fluoxetine occurred via the DA re-uptake mechanism. This method permits a selective measurement of the activity of the DA re-uptake mechanism in brain regions of mixed monoaminergic innervation.

D- and L-AMPH inhibited [^{3}H]-DA accumulation, in the presence of DMI and fluoxetine, in all three DA terminal regions (Fig. 2). D-AMPH was more potent than L-AMPH in each region. The potency of D- and L-AMPH varied slightly between regions, but the ratio of the potencies of D and L isomers was always about 5 (Table 1). This ratio in isomeric potency agrees well with the ratio reported by others for the striatum [5, 6].

A greater potency of D- than L-AMPH also agrees with electrophysiological investigations of nigrostriatal DA neurons [4, 24]. Thus, D-AMPH was 5- to 10-fold more potent than L-AMPH in inhibiting SN DA neuronal impulse flow when given systemically. This inhibition is most likely mediated by release of DA from nerve terminals which activates an inhibitory striatonigral feedback pathway [25]. In contrast, D- and L-AMPH have been reported to be relatively equipotent in decreasing DA impulse flow of VTA DA neurons [24]. However, more recent data (M. K. Sanghera, R. T. Matthews and D. C. German, unpublished observations; [26] have not confirmed this observation. Wang [26] reported that D-AMPH is nine times more potent than L-AMPH on identified mesocortical and mesolimbic DA neurons. Therefore, our data and that of others suggest that mesocortical DA neurons are not different from SN or mesolimbic DA neurons in their electrophysiological and pharmacological response to amphetamine isomers.

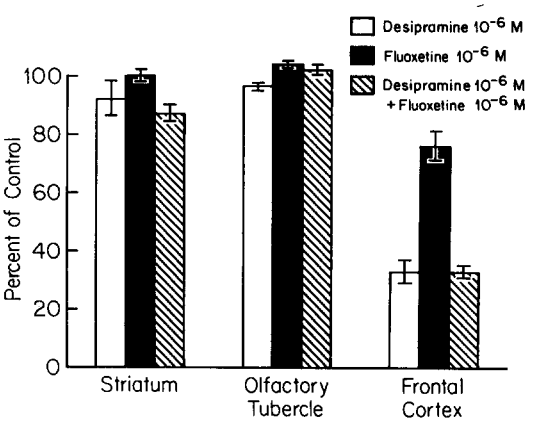

Fig. 1. Inhibition by DMI and fluoxetine of [^{3}H]-DA accumulation by synaptosomal preparations from various brain regions. Each bar represents the mean ± S.E.M. of at least six animals. Average accumulations of [^{3}H]-DA by control samples were 6.8 ± 0.42 (S.E.M.), 3.1 ± 0.17 and 0.11 ± 0.12 pmoles/mg tissue/5 min for striatum, olfactory tubercle, and prefrontal cortex respectively.

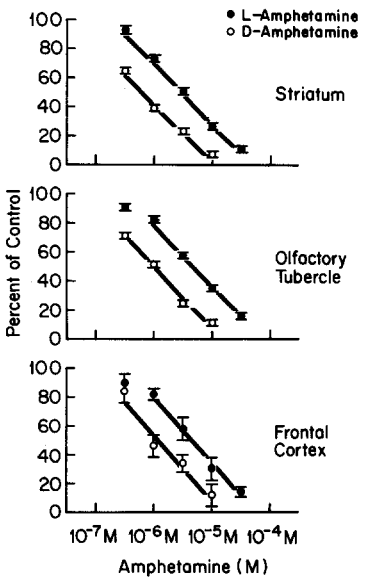

Fig. 2. Inhibition by amphetamine isomers of [^{3}H]-DA accumulation by synaptosomal preparations from various brain regions. All incubations were done in the presence of 10^{-6} M DMI plus 10^{-6} M fluoxetine. Each point represents the mean ± S.E.M. of at least six animals. [^{3}H]-DA accumulations by samples with or without DMI and fluoxetine were not significantly different from data presented in Fig. 1 (Student's *t*-test).

Table 1. IC_{50} Values for inhibition of [^{3}H]-DA accumulation

	IC_{50} (10^{-7} M)*		
	D-AMPH	L-AMPH	Potency ratio L-AMPH/D-AMPH
Striatum	7.0 ± 0.52	33 ± 0.18	4.7
Olfactory tubercle	9.8 ± 0.90	49 ± 0.51	5.0
Prefrontal cortex	11.0 ± 0.94	51 ± 0.81	4.6

* Each value is the mean ± S.E.M., calculated from six (striatum, olfactory tubercle) or ten (prefrontal cortex) dose–response curves, one animal per curve.

In summary, a method for selective measurement of [^{3}H]-DA uptake by the DA neuronal uptake mechanism in synaptosomal preparations of brain areas of mixed monoaminergic terminals was developed. Cortical, but not striatal or olfactory tubercle, synaptosomes showed substantial uptake of [^{3}H]-DA by transport mechanisms sensitive to 5-HT and NE re-uptake blockers. In the presence of DMI and fluoxetine, both amfonelic acid and AMPH isomers inhibited residual DA uptake in all areas. D-AMPH was about 5-fold more potent than L-AMPH in all three DA terminal areas. It now appears that D-AMPH is more potent than L-AMPH in (a) blocking DA re-uptake in terminals of VTA DA neurons, as well as (b) decreasing VTA DA impulse flow and (c) releasing dendritic DA in the VTA.

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Inhibition of high affinity choline transport by stereoisomers of some 3-quinuclidinol derivatives

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The transport of choline (Ch) into cholinergic nerve endings is mediated by a specific, high affinity ($K_T \sim 10^{-6}$ M), Na^+-dependent, hemicholinium-3 (HC-3) sensitive carrier system [1]. The structural demands on substrates for this high affinity Ch transport (HAChT) system are very strict [2]. Inhibitors, however, include both relatively simple analogues of Ch and more complex molecules most of which contain hydrophobic moieties [2, 3]. We have examined the inhibitory effects on HAChT in rat brain synaptosomes of stereochemical isomers of some tertiary amines and quaternary ammonium salts (I–VI, Fig. 1) structurally related to 3-quinuclidinol methiodide, a rigid Ch analogue. Our main interest was to compare the effects of optical isomers since the HAChT system has not been shown previously to display stereochemical selectivity [2]. We also examined the effects of Compounds I–VI on the isolated frog rectus abdominis muscle and on acetylcholinesterase (AChE) *in vitro*.

Materials and methods

Synthesis of new compounds. The enantiomers of 3-quinuclidinol (I), 3-acetoxyquinuclidine hydrochloride (III) and of 3-acetoxyquinuclidine methiodide (IV) were synthesized as previously described [4]. *R*-3-Quinuclidinol methiodide (*R*-II) was prepared by the addition of methyl iodide to a solution of *R*-I in methanol; m.p. 322–324° (from ethanol–ether), $[\alpha]_D^{23} - 32.2°$ (*c* 0.8, 95% ethanol). *S*-3-Quinuclidinol methiodide (*S*-II), prepared similarly, had m.p. 320–323° and $[\alpha]_D^{23} + 32.5°$ (*c* 0.8, 95% ethanol). Anal. Calc. for $C_8H_{16}INO$: C, 35.70; H, 5.99. Found: C, 35.82; H, 6.10.

RR-Bis(3-quinuclidinyl)terephthalate dihydrochloride (*RR*-V) was synthesized from *R*-I [4.0 g, $[\alpha]_D^{23} - 44.6°$ (*c* 2.0, 1 N HCl)], 1.7 M butyl lithium in hexane (25 ml) and 3.35 g of terephthaloyl chloride [5] according to a previously described method [6]. The yield was 4.5 g (74%)