being investigated in more detail in our laboratory.

Source of the methyl group. The results of the double-label experiment designed to confirm the origin of the methyl group in the methylated metabolite are shown in Fig. 2, using [^{3}H-NCH$_3$]-*R*-nicotine as the substrate. Approximately 7.4% of the ^{3}H-activity and 1.1% of the ^{14}C-activity recovered in the column effluent were associated with the *N*-methylated metabolite, indicating transfer of the labeled methyl group from SAM to nicotine. The absence of methylation in the controls proved unequivocally that this process is enzymic.

Again no methylation was observed when [^{3}H-NCH$_3$]-*S*-nicotine was used as the substrate, and hence no incorporation of the ^{14}C-labeled methyl group was seen.

These preliminary studies describe the presence of a methyltransferase system which is widely distributed in guinea pig tissues and which is capable of methylating nicotine to *N*-methylnicotinium ion. The enzyme utilized *S*-adenosyl-L-methionine as co-factor and exhibited specificity for the *R*-isomer of nicotine. Although this type of metabolic reaction was noted many years ago by His [10], and subsequently by others [11–14], it would appear that the *in vivo* methylation of aromatic tertiary amines to water-soluble quaternary metabolites may have been overlooked as a significant route in the biotransformation of heterocyclic drug molecules. We are currently involved in the isolation, purification, and substrate kinetics of the above *N*-methyltransferase enzyme.

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Dopamine microinjection into the nucleus accumbens: correlation between metabolism and behavior

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Many studies have demonstrated that microinjection of dopamine (DA) into the nucleus accumbens of rodents produces an increase in locomotor activity [1–3]. The doses required to produce this effect are in the range of 20–100 nmoles (3–15 μg) DA/accumbens. In contrast, the entire nucleus accumbens contains approximately 0.3 to 0.6 nmole DA [4]. This discrepancy between the dose of exogenous DA required to elicit a behavioral response and the endogenous level of DA has been problematic in interpreting these pharmacological responses in terms of a physiological function for DA in the nucleus accumbens. It is generally assumed that the rapid elimination of injected DA from the synaptic cleft by DA uptake into the presynaptic terminal, degradation to biologically inactive metabolites or diffusion necessitates the use of seemingly non-physiological concentrations of DA.

In this study, we examined the metabolism of [^{3}H]DA which was microinjected into the nucelus accumbens. Further, we evaluated the temporal relationship between the degradation of DA and DA-induced motor activity.

Materials and methods

Male Sprague–Dawley rats were housed individually with food and water made available *ad lib*. When the rats attained a weight of 300–350 g, they were anesthetized with ketamine (80 mg/kg, i.p.) and pentobarbital (10 mg/kg, i.p.), and were stereotaxically implanted with chronic bilateral injection cannulae (26 gauge stainless steel) 1 mm over the nucleus accumbens according to the atlas of Pelligrino *et al.* [5]. The stereotaxic coordinates employed were A/P 9.2, M/L 1.7 and, D/V-0.7 (relative to the interaural line). The range of variation for the nucleus accumbens implantation is described in detail elsewhere [6]. The cannulae were secured in position with set screws and dental acrylic. The rats were then divided into two groups: one group to be used for behavioral measurements (N = 10) and one group for neurochemical analysis (N = 10).

Behavioral measurements were obtained with a photocell apparatus. Each cage, 25 cm × 50 cm × 35 cm high, was equipped with two photocells located 4 cm above the cage floor to estimate locomotor activity. Rats were adapted to the cage for 90 min and then microinjected with either 0.9% (w/v) sterile saline or DA (10 μg/side). Simultaneous bilateral microinjections were made in the unrestrained rat in a volume of 0.5 μl/side over 60 sec with a 33 gauge needle connected via PE-10 tubing to a 1 μl syringe. Immediately after microinjection, the rat was placed in the photocell cage and motor activity was monitored for 90 min. After each rat received one saline and one DA injection in random order with a 72-hr intertrial interval, the rat was killed with ether and the brain was perfused with saline followed by 4% (w/v) paraformaldehyde. The brain was

sectioned with a vibratome, and coronal slices (100 μm thick) containing the cannulae tracks were mounted and stained with cresyl violet. All rats were found to have cannulae positioned correctly in the nucleus accumbens [6].

Neurochemical measurements were made with reversed-phase high pressure liquid chromatography (HPLC) and electrochemical detection [7]. Rats were microinjected as described above with 10 μg DA containing 100,000 dpm of [^{3}H]DA ([2,5,6-^{3}H]dopamine; sp. act. = 13.6 Ci/mmole; Amersham International). Unilateral injections were made at different times, and the rats decapitated at various times after injection. The brain was rapidly removed, and the nucleus accumbens dissected on ice [8]. Each dissected nucleus accumbens was placed in 500 μl mobile phase, sonicated, and centrifuged (11,000 *g* for 3 min). Supernatant fraction (250 μl) was then injected into the HPLC system. Chromatography was performed with a 25-cm stainless steel column packed with octadecysilane, and elution was made with an isocratic mobile phase containing 0.1 M trichloroacetic acid, 0.01 M sodium acetate, 0.01 mM EDTA and 18% (v/v) methanol (pH = 3.8). The flow rate was 2.5 ml/min, and oxidation was performed at +0.7 V. Fractions of 0.7 ml were collected and mixed with 5 ml of scintillation fluid (Ready-Solv EP; Beckman), and radioactivity was measured in a liquid scintillation counter (Beckman; LS 7500). Counting efficiency was determined with an external standard. Cannula placement in this portion of the study was considered correct if the track was observed to penetrate the dorsal but not the ventral surface of the dissected nucleus accumbens [8].

Results and discussion

Figure 1 shows that the recovery of radioactivity corresponded to the HPLC profiles of a standard and tissue sample. Radioactivity was observed only in fractions corresponding to DA and its metabolites. When [^{3}H]DA was added to a tissue homogenate (pH = 3.8), more than 99% of the radioactivity was recovered as [^{3}H]DA, and no radioactivity was recovered as [^{3}H]-3,4-dihydroxyphenylacetic acid ([^{3}H]DOPAC), [^{3}H]homovanillic acid ([^{3}H]HVA) or [^{3}H]-3-methoxytyramine ([^{3}H]3-MT).

When the rats were decapitated immediately after microinjection, more than 95% of the recovered radioactivity was [^{3}H]DA (Fig. 2A). The remaining radioactivity coeluted with either DOPAC or 3-MT, with only one of four rats having any measurable [^{3}H]HVA (Figs. 2B and 2C). Thus, during the maximum 5 min required to remove the brain and dissect and sonicate the nucleus accumbens in mobile phase, measurable metabolism of [^{3}H]DA had occurred. The percent of radioactivity recovered as [^{3}H]DA declined in a linear fashion ($r = -0.940$; N = 16; $P < 0.001$) from 0 to 30 min after microinjection. However, between 30 and 60 min, the percentage of DA recovered appeared asymptotic and declined only slightly. The time course of [^{3}H]DOPAC recovery between 0 and 30 min was inversely correlated with that of [^{3}H]DA ($r = 0.991$; N = 16; $P < 0.001$). Levels of [^{3}H]DOPAC rose rapidly between 0 and 30 min, and then leveled off. The levels of [^{3}H]HVA remained less than 3% of the recovered radioactivity from time 0 to 15 min and then began to increase until [^{3}H]HVA constituted 17.9% of the recovered radioactivity by 60 min after injection. The level of [^{3}H]3-MT was low (0.2 to 0.4%), but it remained relatively constant between 0 and 30 min. However, no [^{3}H]3-MT was detected at 60 min.

The behavioral response to DA microinjection into the nucleus accumbens closely paralleled the linear decline in recovered [^{3}H]DA (Fig. 2D). The motor stimulant effect was maximal between 0 and 30 min after injection and had disappeared by 40–50 min. Since the behavioral response disappeared when approximately 20% of the recoverable radioactivity was [^{3}H]DA, either the concentration of DA remaining in the synaptic cleft was insufficient to elicit a motor response, or the remaining DA had been removed from the synaptic cleft.

The three physiological mechanisms for removing DA from the synaptic cleft are (1) reuptake into presynaptic terminals, (2) diffusion out of the cleft, and (3) degradation of DA by catechol-*O*-methyltransferase to form 3-MT [4, 9]. While these three mechanisms are undoubtedly involved, it was not possible to assess the relative contribution of each mechanism in the present study where a large quantity of exogenous DA has been injected into the nucleus accumbens. Similar to physiological conditions [4, 8, 10], we observed that the majority of microinjected [^{3}H]DA was metabolized to [^{3}H]DOPAC. However, while

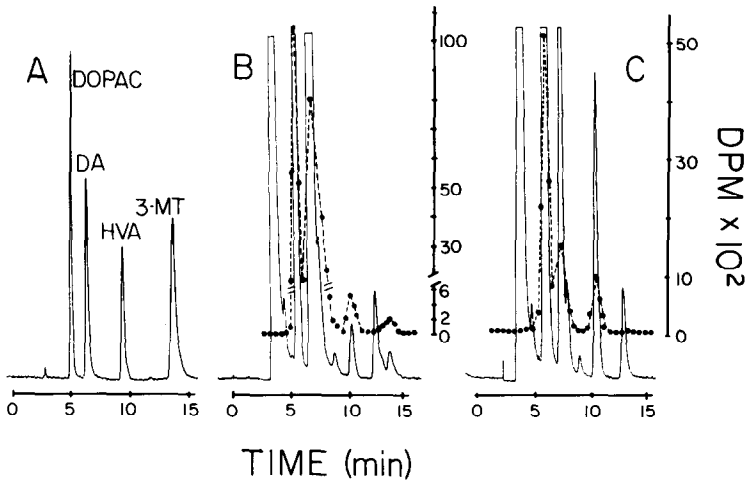

Fig. 1. Comparison of HPLC chromatograms with fractions of recovered radioactivity. (A) Chromatogram standard produced by a mix of 10 ng of each compound. (B) HPLC profile of nucleus accumbens tissue dissected 15 min after injection. The large peak just before 3-MT is serotonin. Note that while the peaks for DOPAC and DA were off-scale, the integrator still obtained a measurement of peak area. (C) HPLC profile of the nucleus accumbens dissected 60 min after injection. Note the relatively large amounts of HVA and DOPAC compared with the chromatogram in Fig. 1B, and the absence of 3-MT.

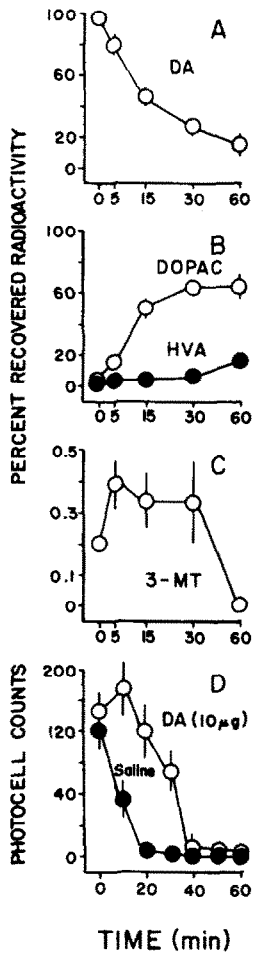

Fig. 2. Comparison of percent radioactivity recovered as DA and its metabolites with the behavioral effect of DA microinjected into the nucleus accumbens. All data are shown as mean ± S.E.M. For the data in A, B and C, N = 4 at each time point. For the behavioral data (D), each rat was given one trial with saline and one with DA in random order (N = 10).

in a physiological situation it is thought that the majority of DOPAC is formed after uptake into the presynaptic terminal, microinjected DA will distribute into both synaptic and nonsynaptic extracellular space making it possible that deamination by monoamine oxidase directed into the extracellular space may have made a significant contribution to the formation of [^{3}H]DOPAC in this study. Under physiological conditions, methylation of DA by catechol-*O*-methyltransferase to form 3-MT occurs almost exclusively outside the neuron, and makes a relatively minor contribution in the inactivation of DA [4, 11, 12]. Likewise, the percentage of radioactivity recovered as 3-MT in this study was low. The level of [^{3}H]3-MT was constant for the first 30 min after microinjection. However, no [^{3}H]3-MT was detected at 60 min, even though the level of recovered [^{3}H]DA declined only slightly between 30 and 60 min. Therefore, at 60 min after injection the microinjected [^{3}H]DA was no longer being metabolized into [^{3}H] 3-MT, presumably because it had either been transported into the presynaptic terminal or had diffused away from the dissected nucleus accumbens.

In conclusion, the time course of the behavioral response produced by the microinjection of DA into the nucleus accumbens is paralleled by a decline in the level of DA. Thus, these data support the rationale that rapid degradation of microinjected DA plays a role in the requirement for the relatively high doses of DA that are necessary to elicit an increase in spontaneous motor activity after microinjection into the nucleus accumbens.

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