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NICOTINE-INDUCED RELEASE OF CATECHOLAMINES FROM RAT HIPPOCAMPUS AND STRIATUM*

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Abstract—The present report is a comparative study of [^{3}H]catecholamine release from noradrenergic and dopaminergic neuron terminals of the central nervous system, induced by nicotine and potassium depolarization. Striatal and hippocampal slices of rat brain that had incorporated [^{3}H]catecholamines in previous incubations with the radioamines were superfused and stimulated by nicotine and high potassium. Nicotine produced a marked [^{3}H]catecholamine release from these two brain areas, this effect being much greater in the striatum. The time course of radioactive efflux released by nicotine was different from that induced by high potassium and similar to that evoked by tyramine. Nicotine induced released was dose dependent, inhibited by low temperature, independent of extracellular calcium and not inhibited by an excess of magnesium. Studies with newly synthesized [^{3}H]dopamine and [^{14}C]urea suggested that nicotine acted on the storage vesicles. [^{3}H]catecholamine release was continuous during prolonged nicotine stimulation, in contrast to the transient efflux induced by prolonged stimulation by potassium, and additive when the slices were superfused simultaneously with nicotine plus potassium. Previous nicotine superfusion did not modify the typical potassium release, and the response to nicotine was also not altered when the slices were exposed previously to high potassium. Based on these results, the mechanism of nicotine release of catecholamines from central catecholaminergic neuron terminals is discussed.

Nicotine produces release of catecholamines from central and peripheral adrenergic neurons and chromaffin cells [1–5]; it also has been found to lower the content of catecholamines and increase the turnover of noradrenaline in the brain [6, 7]. The pharmacological central effects of nicotine are related to their releasing action of monoamines from central catecholaminergic neurons. However, relatively few studies have been published on the mechanism of catecholamine release by nicotine from central neurons. Westfall [5] has postulated that nicotine-releasing action in the central nervous system is related to a direct effect on nicotinic presynaptic receptors, but nicotine might very well be acting by displaying monoamines from their storage sites.

In search of support for these proposals, we studied the mechanism by which nicotine releases monoamines from central catecholamine-containing neurons. Previous studies have shown that nicotine may release catecholamines from slices of brain hypothalmus, cortex and striatum [4, 5]. For our studies, we have chosen brain areas such as the hipocampus and striatum, each of them rich in noradrenergic and dopaminergic nerve terminals, respectively, and we compared, using a superfusion system, the release of monoamines induced by nicotine and by potassium depolarization.

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MATERIALS AND METHODS

Preparation and incubation of tissue slices. Male Sprague–Dawley rats weighing between 100 and 150 g were decapitated, the whole brain was carefully removed, and the striatum and hippocampus were dissected as described by Glowinki and Iversen [8]. Striatal and hippocampal tissue slices (0.20 mm in thickness) were prepared with a Sorvall tissue chopper. The release of labeled catecholamines was followed from the striatum and hippocampus in a way similar to that described by Bustos and Roth [9]. Four slices (6–7 mg wet wt) of hippocampus or striatum tissue were incubated for 30 min at 37° in 2 ml of Krebs–Ringer Phosphate (KRP), pH 7.4, saturated with 95% O_2 + 5% CO_2 and containing either L[3,5-^{3}H]tyrosine (sp. act. 69.36 Ci/m-mole, final concn 2×10^{-7} M), DL[7-^{3}H]norepinephrine (sp. act. 9.3 Ci/m-mole, final concn 8×10^{-7} M) or [^{3}H]-dopamine (3,4-dihydroxyphenylethylamine[ethyl-2-[^{3}H](N)], sp. act. 7.5 Ci/m-mole, final concn 6×10^{-7} M).

Release of labeled catecholamines. The slices, after incubation with the radioamines, were then transferred to superfusion chambers from which the release of newly formed or exogenously taken up [^{3}H]NE or [^{3}H]DA followed. The superfusion chamber was maintained at 37° by a heated water jacket. The slices were washed with 10 ml KRP and then superfused at a constant flow rate of 4 ml/min with KRP solution which was being continuously oxygenated and prewarmed to 37°. An initial superfusion period of

10 min was allowed before stimulus-induced release. Two samples of 1 min each were then collected to determine the spontaneous release of catecholamines. Stimulated release from striatal slices was induced by changing the superfusion solution for 1 min to KRP containing either K^+ (53 mM), nicotine (5 or 10 mM) or tyramine (0.5 mM). Thereafter, the superfusion fluid was changed back to normal KRP, and four additional samples of 1 min each were collected. The slices were then superfused for a further 10-min period with KRP solution before the next stimulation was induced. When studying release of monoamines from hippocampus, the slices were stimulated for 2 min, and four additional samples were collected for a period of 6 min after stimulation. At the end of the superfusion period, catecholamines were extracted from tissue slices with trichloroacetic acid (TCA) (15%, w/v) and centrifuged at 12,000 *g* for 10 min. The release of newly formed [^{3}H]DA was studied essentially in the same way as described above for the release of exogenous labeled catecholamines. Samples containing the released material were collected every min in tubes containing 1 ml TCA (50%, w/v) and carrier DA (50 μg). All the released material and supernatant fractions obtained from homogenized tissue slices were immediately frozen and kept for adsorption chromatographic analysis.

Separation of labeled dopamine by column chromatography. Separation of [^{3}H] catechols from [^{3}H]tyrosine and deaminated metabolites was carried out by adsorption chromatography through alumina columns as described by Bustos and Roth [9]. The recovery of catecholamines by this procedure was 81 per cent; reported values have not been corrected for recovery. The labeled released material was identified to be more than 50 per cent unmetabolized NE or DA when further analyzed by ion exchange chromatography on Dowex 50. When the release of exogenous labeled catecholamines was reported, the total output of radioactivity was referred to as dopamine or noradrenaline release.

Determination of radioactivity. An aliquot of 1 ml of effluent superfusate or eluate from the column was added to 15 ml of a scintillation mixture (2:1 mixture of toluene containing 4 g PPO* and 0.1 g POPOP/l, and Triton X-100) and measured in a Mark I scintillation counter.

Analysis of the data. The release of catecholamines was expressed as a percentage of the total radioactivity present in the tissue before stimulation. The total radioactivity present in the tissue before stimulation was determined by substracting, from the total label present in the tissue (radioactivity present in all the superfusate effluent plus the radioactivity present in the supernatant fraction of the homogenized tissue slices), the radioactivity present in all the superfusate effluent collected before stimulation. The induced release was calculated by substracting the estimated spontaneous radioactivity released from the total radioactivity released for each fraction during stimulation and after 3 min. The estimated spontaneous release was calculated assuming a linear decline of the latter with a 1-min interval before the stimulation period. Results are expressed as mean $\pm$ S. E. M., and Student's *t*-test was used for comparison of mean values. The number of experiments is indicated by N.

Solutions and chemicals. The Krebs–Ringer Phosphate (KRP) solution used had the following composition: NaCl, 128 mM; KCl, 4.8 mM; $CaCl_2$, 0.75 mM; $MgSO_4$, 1.20 mM; glucose, 16 mM; and Na_2HPO_4, 16 mM at pH 7.4. Krebs–Ringer Phosphate high K^+ (KRP-high K^+) was made by replacing part of the NaCl with equimolar amounts of KCl. When calcium was not added to the KRP (Ca^{2+}-free KRP) or excess magnesium was added to the KRP [(final concn 24 mM (high Mg^{2+}-KRP)], the osmolarity was not corrected. L[3,5-^{3}H]tyrosine (30–50 Ci/m-mole); [^{3}H]-dopamine (5–10 Ci/m-mole); DL[7-^{3}H]norepinephrine (5–15 Ci/m-mole); and [^{14}C]urea (40–60 mCi/m-mole) were obtained from New England Nuclear Corp., Boston, M.A. Nicotine and tyramine hydrochloride and aluminium oxide were purchased from BDH Chemicals Ltd., Poole, England. Dopamine and norepinephrine were obtained from Sigma Chemical Co., St. Louis, MO.

RESULTS

Effect of repeated high potassium, nicotine or tyramine stimulation on [^{3}H]efflux from the striatum. Stimulation by high potassium, nicotine or tyramine produced a sharp increase in the efflux of radioactivity into the superfusate, being, respectively, 12.81 $\pm$ 0.19-, 11.19 $\pm$ 1.91- and 26.67 $\pm$ 5.10-fold over the prestimulation period (Fig. 1). The percentage of radioactivity released during 1 min before the stimulus amounted to 2.13 $\pm$ 0.03; 1.43 $\pm$ 0.07; and 1.21 $\pm$ 0.14 (mean $\pm$ S. E. M., N = 3) for the potassium, nicotine and tyramine experiments respectively. The time course of efflux of radioactivity induced by nicotine was very similar to the efflux evoked by tyramine, where the larger proportion of radioactivity was released after the stimulation period. In contrast, high potassium caused an almost immediate rise in the efflux of radioactivity, and the totality of [^{3}H]DA was released during the stimulation period. High potassium, nicotine and tyramine induced fairly constant and reproducible responses when successive stimulations occurred at 12-min intervals.

Release of [^{3}H]catecholamines from striatum and hippocampus induced by increasing concentrations of nicotine and tyramine. The effects of increasing concentrations of the drugs on the ability to release [^{3}H]-monoamines from striatal and hippocampal slices are shown in Fig. 2. These data show that induced release of [^{3}H]DA by tyramine was considerably greater and more sensitive than that obtained with nicotine, and the slope of the dose–response curve was much steeper. It can be seen that nicotine-induced release of [^{3}H]NE was markedly greater than that evoked by tyramine from hippocampus.

Effect of temperature on nicotine-induced release of catecholamines. The effect of temperature on the release of [^{3}H]monoamine from the striatum and hippocampus is shown in Table 1. When the temperature of the superfusion medium was lowered below 6°, spontaneous and nicotine-induced releases decreased markedly, returning to previous values when the temperature was increased again to 37°, showing,

* PPO, 2,5-diphenyloxazole; POPOP, 14-bis-[2-5-phenyloxazolyl)]benzene.

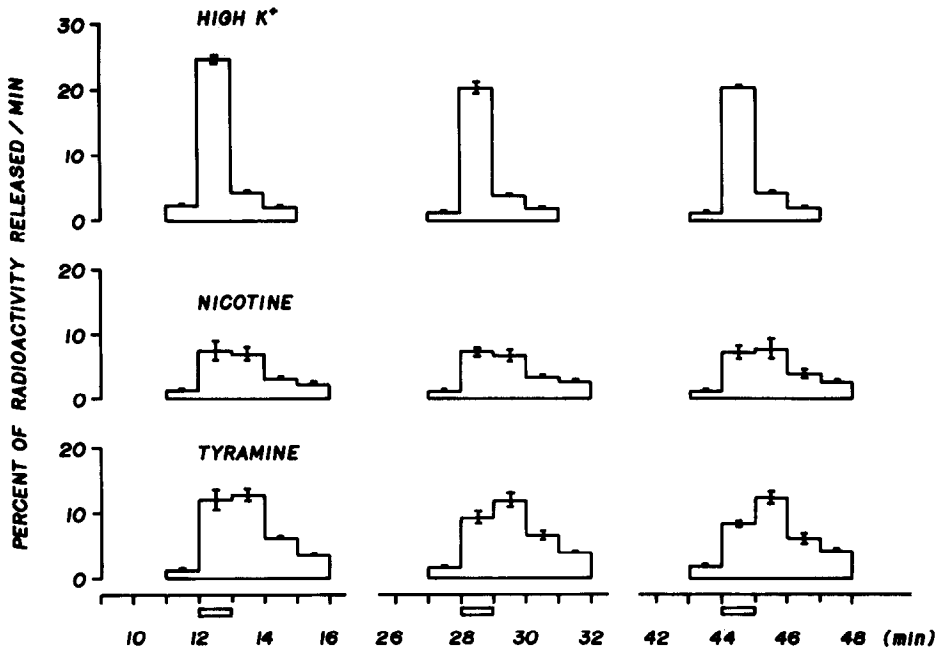

Fig. 1. Time course and repeated induced release of [^{3}H]DA by high potassium, nicotine or tyramine. Striatal slices were incubated with [^{3}H]DA for 30 min prior to being placed in superfusion chambers as described in Materials and Methods. On the abscissa is plotted the superfusion time and on the ordinate is the percentage of radioactivity released per min of superfusion. Each horizontal bar represents the period of stimulation when potassium, nicotine or tyramine was administered. Each stimulant was superfused three times at intervals of 16 min. The tissue took up 1,644,100 ± 253,838; 1,297,500 ± 134,555; and 1,366,400 ± 122,806 cpm of [^{3}H]DA for the potassium, nicotine and tyramine experimental groups respectively. Each graph shows the mean ± S. E. M. for N = 3.

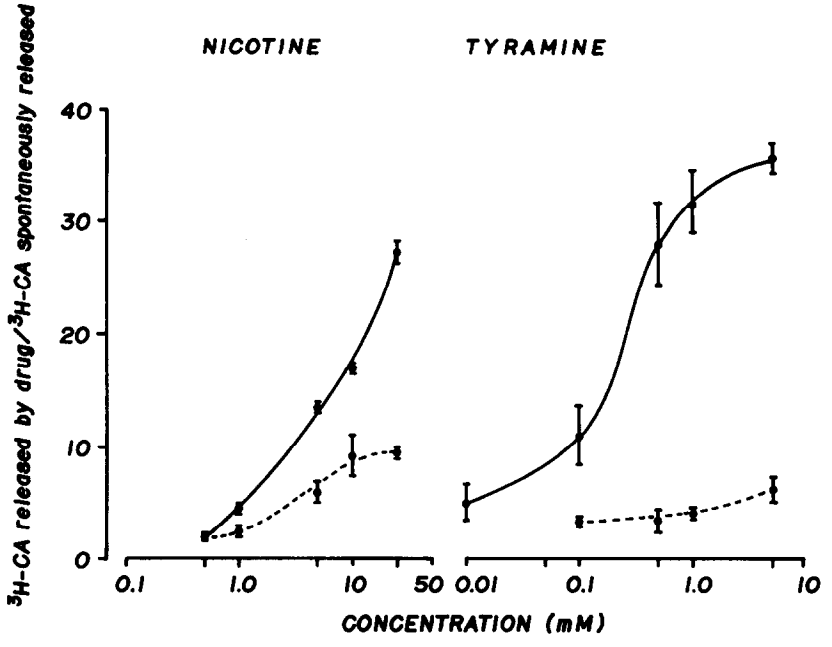

Fig. 2. Dose–response curves showing the effect of increasing concentrations of nicotine and tyramine on the release of [^{3}H]catecholamines from striatum (——) and hippocampus (. . .). Striatal and hippocampal slices were incubated with [^{3}H]DA and [^{3}H]NA, respectively, and then transferred to superfusion chambers, as described in Materials and Methods. Nicotine and tyramine stimulation periods were carried out after an initial superfusion of 12 min. Concentration of nicotine or tyramine is plotted on the abscissa and the ratio of induced release/spontaneous release on the ordinate. Spontaneous release was 1.11 ± 0.01 and 1.10 ± 0.10 per cent of radioactivity present in hippocampal and striatal slices, respectively, before stimulation. Each point represents the mean of at least four experiments, and vertical bars represent the standard error of the mean.

Table 1. Effect of temperature on nicotine-induced release of [^{3}H]catecholamines from striatum and hippocampus*

Temperature	Release before stimulation (per cent of [^{3}H]DA released)	Release during stimulation (per cent of [^{3}H]DA released)	Release before stimulation (per cent of [^{3}H]NE released)	Release during stimulation (per cent of [^{3}H]NE released)
37°	1.78 ± 0.37	11.64 ± 1.00	2.37 ± 0.37	10.40 ± 1.23
6°	0.34 ± 0.03	0.60 ± 0.18	0.35 ± 0.06	1.37 ± 0.39
37°	1.16 ± 0.07	13.72 ± 0.50	1.03 ± 0.22	9.14 ± 1.42

* Striatal and hippocampal slices were incubated for 30 min at 37° in the presence of [^{3}H]catecholamines as described in Materials and Methods. Release of [^{3}H]catecholamines was then measured in normal KRP at 37, 6 and 37 again. Between the temperature changes the slices were superfused for 10 min with the colder or warmer solution. The release of [^{3}H]catecholamines was calculated by adding the radioactivity released during stimulation period plus the 3-min period immediately after and subtracting from this value the estimated spontaneous release during 4-min period. The tissues took up 1,535,333 ±196,132 [^{3}H]DA and 179,466 ± 25,934 [^{3}H]NE cpm, and the values for the spontaneous releases were 26,759 ± 1373 and 2509 ± 251 cpm of [^{3}H]DA and [^{3}H]NE respectively. The striatal slices were stimulated with nicotine (5 mM). Results represent the mean ±S. E. M. of three different experiments.

therefore, that both spontaneous and induced releases by nicotine were temperature dependent.

Spontaneous and nicotine-induced release of newly synthesized [^{3}H]dopamine from the striatum. To determine the nicotine-induced release of newly formed [^{3}H]DA, striatal slices were incubated with [^{3}H]tyrosine and then transferred to a superfusion system in which the release of newly formed [^{3}H]DA was followed. It can be seen that 1-min of nicotine stimulation produced a marked increase in the release of newly synthesized dopamine (Fig. 3). A similar response was obtained after a second stimulation period by nicotine. The time course of nicotine-induced release of newly synthesized or exogenously taken up [^{3}H]dopamine was very similar (Figs. 1 and 3).

Effect of Ca^{2+} and Mg^{2+} ions on high potassium and nicotine-induced release of exogenous [^{3}H]dopamine from the striatum. In support of the previous observation of Bustos and Roth [9], the omission of Ca^{2+} inhibits the release of [^{3}H]DA induced by high potassium. After 10 min with a superfusion Ca^{2+}-free KRP solution, high potassium elicited only a very small response (Table 2). This effect was reversed after introducing Ca^{2+} to the superfusion medium. However, removal of Ca^{2+} from the superfusion medium had no effect on the release of [^{3}H]DA induced by nicotine. Table 2 also shows that Ca^{2+}-free KRP plus EGTA (10^{-4} M) or KRP with excess Mg^{2+} (24 mM) inhibited the high potassium induced release; however, the nicotine-induced release was not altered under these experimental conditions.

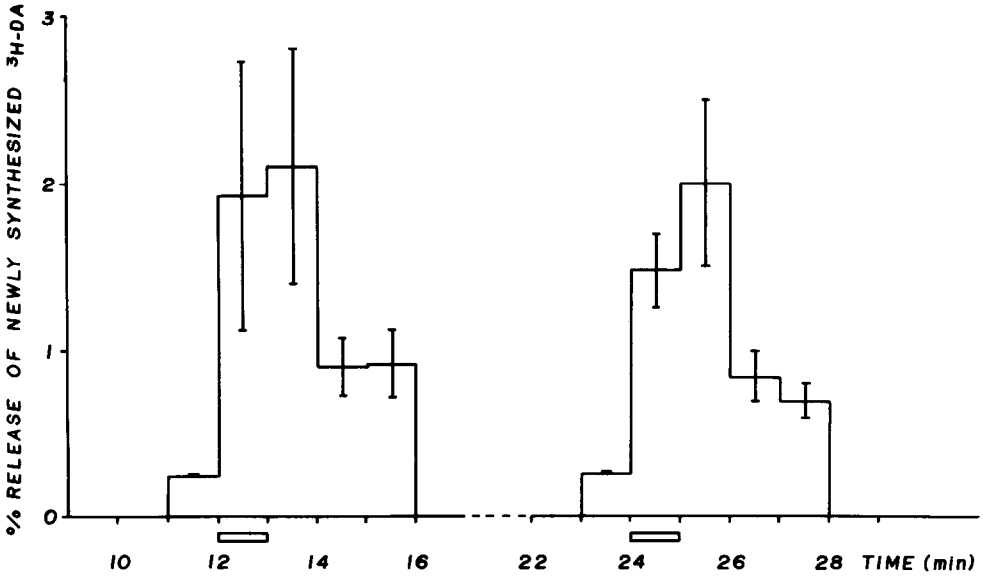

Fig. 3. Nicotine-induced release of newly synthesized [^{3}H]dopamine from striatum. Striatal slices were incubated with [^{3}H]tyrosine (2×10^{-7} M) for 30 min at 37°. [^{3}H]dopamine was separated by column chromatography analysis. On the abscissa the superfusion time is plotted and on the ordinate the percentage of radioactivity released. Nicotine stimulation is skown by a horizontal bar and it is maintained for 1 min after a washout period of 12 min. The tissue had synthesized an average of 357,016 ± 39,444 cpm of [^{4}H]DA. Values shown represent the mean of three different experiments, and vertical bars represent the standard error of the mean.

Table 2. Effect of Ca^{2+} on K^+ and nicotine-induced release of [^{3}H]dopamine from striatum*

	Release of [^{3}H]dopamine (% of radioactivity released)			
	Normal KRP	Ca^{2+}-free KRP	Ca^{2+}-free + EGTA–KRP	High Mg^{2+}–KRP
Spontaneous release	1.60 ± 0.21 (12)	1.91 ± 0.30 (3)	1.56 ± 0.31 (3)	0.54 ± 0.02 (3)
K^+-induced release	26.21 ± 1.24	4.36 ± 0.57†	4.89 ± 2.01†	2.71 ± 0.28†
Spontaneous release	1.21 ± 0.11 (12)	1.85 ± 0.30 (3)	0.91 ± 0.12 (3)	0.62 ± 0.16 (3)
Nicotine-induced release	18.51 ± 1.51	23.19 ± 2.08	16.92 ± 0.55	15.13 ± 0.81

* Striatal slices were incubated for 30 min at 37° in the presence of [^{3}H]DA. Release was then measured in a superfusion chamber, as described in the text. The response to potassium (53 mM) and nicotine (10 mM) was measured in normal KRP, Ca^{2+}-free + EGTA (10^{-4} M) KRP and high magnesium (24 mM)–KRP. The stimulation periods were carried out for 1 min and values shown represent the mean ± S. E. M. Spontaneous and induced release was calculated as described in Table 1. The tissue took up 1,356,477 ± 94,135 and 1,435,955 ± 104,605 cpm of [^{3}H]DA for the potassium and nicotine experimental groups respectively. The values for spontaneous release were 18,580 ± 2429 cpm in normal KRP, and 18,327 ± 1528 cpm in Ca^{2+}-free or high Mg^{2+} KRP conditions. The number of experiments is indicated in parentheses.

† $P < 0.001$ when compared to respective normal KRP-induced release.

The specificity of high potassium- and nicotine induced release of [^{3}H]catecholamines was studied by incubating striatal slices with [^{14}C]urea. Nicotine and potassium stimulation caused a significant increase in the release of [^{3}H]DA without affecting the [^{14}C] urea efflux from striatal slices, under similar experimental conditions (Fig. 4.)

Effect of Ca^{2+} *on high potassium- and nicotine-induced release of exogenous* [^{3}H]*norepinephrine from hippocampus.* The high potassium-induced release of previously taken up [^{3}H]NE into hippocampal slices was shown to be dependent on the presence of Ca^{2+} in the medium (Table 3). Potassium and nicotine stimulation periods produced, respectively, almost 10- and 9-fold increases in the release of [^{3}H]NE over the prestimulation periods. When hippocampal slices were superfused with Ca^{2+}-free KRP plus EGTA (10^{-4} M), potassium depolarization elicited

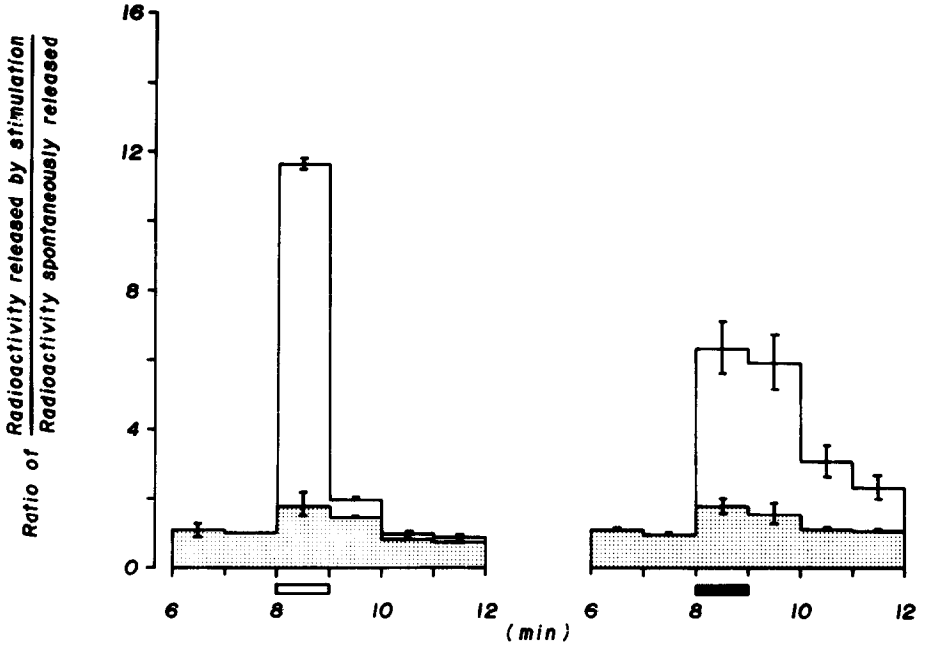

Fig. 4. Release of [^{14}C]urea (shaded columns) and [^{3}H]dopamine (open columns) from striatum induced by potassium and nicotine. Tissue slices were incubated in the presence of 10 μCi [^{14}C]urea or [^{3}H]dopamine as described in Materials and Methods. The values for the ratios of radioactivity released by stimulation and spontaneously released are plotted on the ordinate versus time of superfusion on the abscissa. The horizontal bars indicate the stimulation period by potassium (▭) and nicotine (▬), after a washout period of 6 min. The values for the spontaneous releases were 22,060 ± 4090 cpm of [^{3}H]DA and 658 ± 53 cpm for [^{14}C]urea (N = 6). Each point represents the average of three experiments, and verticals bars indicate the standard error of the mean.

Table 3. Effect of Ca^{2+} on K^+ and nicotine-induced release of [^{3}H]noradrenaline from hippocampus*

	Release of [^{3}H]noradrenaline (% of radioactivity released)		
	Normal KRP	Ca^{2+}-free KRP	Ca^{2+}-free + EGTA-KRP
Spontaneous release	1.06 ± 0.06 (9)	1.90 ± 0.11 (3)	1.95 ± 0.04 (3)
K^+-induced release	11.55 ± 1.03	3.55 ± 0.20†	4.21 ± 0.66†
Spontaneous release	1.09 ± 0.14 (15)	1.48 ± 0.12 (3)	1.80 ± 0.22 (7)
Nicotine-induced release	9.19 ± 0.89	11.13 ± 1.02	11.12 ± 0.62

* Hippocampus slices were incubated in the presence of [^{3}H]NE, prior to being placed in superfusion chambers, as described in the text. Spontaneous and induced release was calculated as described in Table 1. The response to potassium and nicotine was measured in normal KRP, Ca^{2+}-free KRP and Ca^{2+}-free + EGTA (10^{-4} M)-KRP. The tissue took up 284,592 ± 19,674 and 226,438 ±19,950 cpm of [^{3}H]NE for the potassium and nicotine experimental groups respectively. The values for spontaneous release were 2334 ± 162 in normal KRP and 2414 ± 182 cpm in both Ca^{2+}-free KRP conditions. Values shown represent the mean ±S. E. M. The number of experiments is indicated in parentheses.

† $P < 0.01$ when compared to respective normal KRP-induced release.

only a very small release in contrast with the marked release produced by nicotine (Table 3). The nicotine-induced release of catecholamines from the hippocampus and striatum was shown to be independent of the presence of extracellular calcium.

Effect of a prolonged period of stimulation by potassium and nicotine on the release of [^{3}H]*dopamine from the striatum.* The effect of a prolonged period of stimulation by potassium and nicotine is shown in Fig. 5. Potassium and nicotine produced different patterns of efflux of radioactivity. Nicotine produced a marked increase in the release of [^{3}H]DA, immediately reaching a peak which was sustained during the period of stimulation. Potassium induced a fast and marked

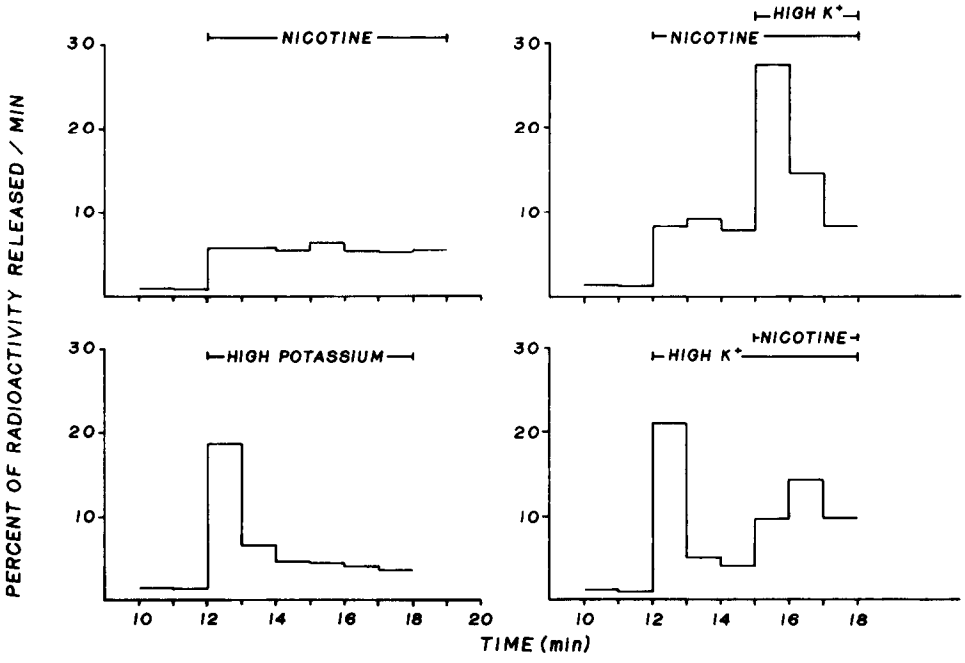

Fig. 5. Effect of prolonged and overlapping periods of stimulation by K^+ and nicotine on the release of exogenous [^{3}H]DA. Striatal slices were incubated with [^{3}H]DA and superfused as described in Materials and Methods. Slices were exposed to K^+ for 6 min and to nicotine for 7 min after a washing period of 12 min (left figures). Potassium and nicotine were added simultaneously 3 min after the start of the prolonged superfusion period with nicotine or K^+, respectively (right figures). The percentage of radioactivity released is plotted on the ordinate and the time of superfusion on the abscissa The K^+ or nicotine stimulation period is indicated by a dark line at top of figures. Values shown represent the mean of two experiments for each figure. The tissue had taken up 885, 170 ± 46,224 cpm of [^{3}H]DA for eight different experiments.

increase in the release of [^{3}H]DA with a rapid return to spontaneous levels in spite of the stimulus still being present. Figure 5 also shows the effect of potassium and nicotine on [^{3}H]dopamine release when they were added, respectively, to striatal slices which had been previously stimulated with nicotine or potassium (Fig. 5). Striatal slices, which had released [^{3}H]DA in response to nicotine stimulation, were still able to respond to potassium stimulation. Interestingly enough, the potassium-induced release was not modified by the response evoked by nicotine. Similarly, the response to nicotine was not appreciably modified when the slices had previously been exposed to high potassium (Fig. 5). In this last case, nicotine produced a marked release of [^{3}H]DA when the efflux induced by potassium had returned to spontaneous levels.

DISCUSSION

The present experiments have shown that nicotine causes a significant increase in the efflux of labeled catecholamines from hippocampus and striatum when it is used for short periods of stimulation (Fig. 1). The responses induced by nicotine, tyramine and high potassium are very reproducible and constant. The release of catecholamines induced by tyramine under our conditions (Fig. 1) is very similar to the data reported by von Voigtlander and Moore [10] using a cerebroventricular perfusing method. However, the time course of efflux of radioactivity evoked by nicotine is different from that induced by high potassium, but very similar to the response evoked by tyramine. Similar results were obtained using hippocampal slices (results not shown). These data suggest that nicotine causes the release of catecholamines from monoaminergic terminals, located in the hippocampus and striatum, by a different mechanism from that induced by potassium depolarization.

Nicotine and tyramine released labeled noradrenaline and dopamine from rat hippocampus and striatum in a dose-related way. However, the releasing effect on striatum was much greater than on the hippocampus. The induced release of catecholamines from hippocampus and striatum was more sensitive to tyramine than to nicotine (Fig. 2), but nicotine was more effective than tyramine in inducing release of catecholamines from hippocampus. In contrast, tyramine was more effective than nicotine in evoking [^{3}H]dopamine release from striatum. These data are consistent with the preferential accumulation of [^{14}C]nicotine by hippocampus [11].

In order to determine the mechanism of release by nicotine, it is necessary first to demonstrate the site at which nicotine acts to induce release of [^{3}H]catecholamines. When tissue slices were incubated with [^{14}C]urea or [^{3}H]tyrosine, nicotine did not increase the efflux of [^{14}C]urea (Fig. 4) but markedly induced the release of newly synthesized [^{3}H]dopamine (Fig. 3). These results were consistent with the idea that [^{3}H]dopamine release cannot be explained simply by an increase in diffusion or a change in cell permeability, and that the newly formed [^{3}H]dopamine most likely was coming from storage vesicles located in the monoaminergic neuronal terminals. Moreover, the fact that induced release by nicotine and tyramine showed a constant and reproducible response (Fig. 1) indicated that the release of [^{3}H]catecholamines cannot be related to passive displacement from extravesicular or extraneuronal binding sites. In agreement with these results, nicotine did not induce the release of exogenously taken up [^{3}H]dopamine from striatal slices obtained from reserpinized rats (L. Arqueros *et al.*, unpublished data).

The time course of nicotine-induced release of [^{3}H]dopamine, previously taken up or endogenously synthesized from [^{3}H]tyrosine by dopaminergic terminals, presented a similar pattern (Figs. 1 and 3). However, the release induced by nicotine was 4 per cent of the total newly formed [^{3}H]dopamine as compared to 14 per cent of the total exogenously taken up [^{3}H]dopamine. These results are similar to those found by Bustos and Roth [9], who demonstrated that potassium induces release of [^{3}H]dopamine newly formed and taken up by striatal slices. On the other hand, the time course of nicotine-induced efflux of newly synthesized [^{3}H]dopamine (Fig. 3) was different from that evoked by potassium depolarization (Fig. 3, [9]).

Our results show clearly that the catecholamine release evoked by nicotine from hippocampus and striatum is a temperature-dependent process. Accumulation of [^{14}C]nicotine by brain slices has also been shown to be affected by low temperature [12, 13]. Although the metabolic energy may be used for the intracellular accumulation of nicotine, it is not known whether it is also necessary for the release of endogenous catecholamines from the storage complex located in the vesicles. If catecholamines can be replaced by nicotine, and nicotine induces release of catecholamines by displacement from the storage vesicles, then nicotine should be released by potassium depolarization or nicotine stimulation. In preliminary experiments, we found that [^{3}H]nicotine accumulated by striatal slices was released by potassium and nicotine stimulation, and that [^{3}H]nicotine released, either by potassium or nicotine, was inhibited by pretreatment with reserpine (L. Arqueros *et al.*, unpublished data).

The mechanism of release of catecholamines from peripheral noradrenergic nerves induced by electrical stimulation or depolarizing agents is dependent on the extracellular calcium present. This dependency may be related to an increase of calcium influx triggering the catecholamine release process [14–17]. In contrast to the excitation–secretion process, the sympathomimetic amine tyramine releases catecholamines independently of extracellular calcium from noradrenergic vesicles storage and does not involve the exocytosis process [18–21]. Study of the calcium requirement demonstrated that nicotine-induced release, in contrast to that evoked by potassium depolarization, does not depend on extracellular calcium. Excess magnesium ions or the absence of calcium with EGTA markedly inhibited the release induced by potassium, whereas the nicotine-evoked release was not affected. On the other hand, our results disagree with the previous report [5] of extracellular calcium dependency for nicotine-induced release of tritiated norepinephrine from hypothalamus. Consistent with our findings was the fact that the nicotine-induced efflux of [^{14}C]-5-hydroxytryptamine was not altered

by the absence of calcium and that the effect induced by potassium was inhibited when calcium was removed [22].

Finally, the additive responses evoked by potassium plus nicotine stimulation (Fig. 5), the different pattern of response induced by prolonged exposure to potassium or nicotine, and the response evoked by nicotine, even when the release of [^{3}H]DA by potassium was no longer effective (Fig. 5), can be explained only by a different mechanism of release.

It can be concluded that potassium depolarization and nicotine cause a release of monoamines in the central nervous system by a different mechanism. Nicotine effects may be explained by a displacement of monoamines from the vesicle storage complex, not dependent on extracellular calcium but similar to the indirect action of sympathomimetic amines in peripheral noradrenergic nerve terminals. This mechanism of action may explain, in part, some of the already known central effects of nicotine and its pharmacological similarities to amphetamine central effects [23].

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