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European Journal of Pharmacology 341 (1998) 23–32

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The effects of nicotine on dopamine and DOPAC output from rat striatal tissue

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Received 26 June 1997; revised 15 September 1997; accepted 14 October 1997

Abstract

The effects of varying doses of nicotine infusion upon spontaneous (basal) and subsequent potassium chloride-stimulated dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) output from superfused corpus striatal tissue fragments of male rats were tested. Spontaneous dopamine and DOPAC outputs were increased in response to 1, 5 and 10, but not to 0.1 and 0 (control) μM concentrations of nicotine. Interestingly, the subsequent K^+-stimulated (30 mM) dopamine output was completely abolished in preparations infused with the 5 and 10 μM nicotine, but not with the 1 or 0.1 μM nicotine. No overall significant differences in K^+-stimulated DOPAC were obtained among the five doses. In experiment 2, the effects of an initial infusion of amphetamine (10 μM), potassium chloride (30 mM), nicotine (10 μM) or normal superfusion medium (control) were compared upon subsequent K^+-evoked dopamine release. The amount of dopamine released in response to the second (subsequent) infusion of K^+ was significantly greater in the potassium chloride and control conditions versus the nicotine and amphetamine stimulated groups. No overall differences in DOPAC output were observed among the four conditions of experiment 2. These results demonstrate that nicotine can exert differential modulatory effects upon striatal dopaminergic activity as a function of the dose. The augmented levels of DOPAC output along with the abolition of the K^+-stimulated dopamine release in response to the 5 and 10 μM nicotine doses suggest that these doses may simultaneously produce an activation of intraneuronal metabolism of dopamine to DOPAC along with an activation of release and inhibition of uptake to diminish stores available for subsequent responses to K^+ stimulation. © 1998 Elsevier Science B.V.

Keywords: Nigrostriatal; Amphetamine; Dopamine uptake; Dopamine metabolism; Dopamine release

1. Introduction

Nicotine has attracted considerable attention of late, with both positive and negative attributes being associated with this drug. On the positive side are reports indicating that nicotine may serve as an aid for smoking cessation (Balfour and Fagerstrom, 1996), protect against neuronal cell death (Socci and Arendash, 1996; Yamashita and Nakamura, 1996), improve memory performance (Levin and Torry, 1996) and be beneficial in the prevention/delay of neurodegenerative disorders like Alzheimer's and Parkinson's disease (Janson et al., 1989; Nordberg et al., 1989; Morens et al., 1995; Balfour and Fagerstrom, 1996). On the negative side are the addictive properties of this agent resulting from its apparent capacity to serve as a substance capable of generating augmented levels of extracellular dopamine within specific dopaminergic systems (Ritz and Kuhar, 1993; Stolerman, 1993; Pich et al., 1997).

The nigrostriatal dopaminergic system represents one important central nervous system target site for the actions of nicotine. A variety of techniques have been used to assess the effects of nicotine upon this dopaminergic system including measurements of dopamine metabolism (Haikala et al., 1986; Liekola-Pelho et al., 1990; Tsai and Lee, 1995), uptake (Izenwasser et al., 1991; Izenwasser and Cox, 1992) and in vivo release (Imperato et al., 1986; Wonnacott et al., 1996). Most methods of assessing nicotine's effects upon the nigrostriatal dopaminergic system have employed in vitro superfusion of [^{3}H]dopamine with the majority of these using striatal synaptosomal preparations (Rapier et al., 1988; Rowell and Hillerbrand, 1994; Rowell, 1995; Whiteaker et al., 1995). Others have used superfusion with striatal slices (Sacaan et al., 1995) or both slice and synaptosomal preparations (Izenwasser et al., 1991; Marshall et al., 1996). Results obtained from these

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two preparations are not always comparable due to the differing degrees of integrity and resultant mechanistic features associated with each technique (Izenwasser et al., 1991). In the present report, endogenous, unlabeled levels of both dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) outputs were measured in perfusate samples from superfused corpus striatal tissue fragments infused with varying doses of nicotine. There were two major goals that comprised the present experiments. First, in nearly all previous reports [^{3}H]dopamine and no DOPAC measurements were performed. Therefore, with the simultaneous measurements of endogenous dopamine and DOPAC output it should be possible to achieve a more complete evaluation of the interactive effects of nicotine upon release, uptake and metabolism processes. As an additional assessment of nicotine's actions upon striatal dopaminergic function the potassium chloride stimulated dopamine and DOPAC release rates were measured in these preparations. With these determinations the potential effects of nicotine upon vesicular storage sites of dopamine which are activated by potassium chloride can be evaluated. Second, the relatively more intact nature of the corpus striatal tissue fragment preparation may permit a better assessment of overall functional activity of the corpus striatum in response to nicotine (Marshall et al., 1996).

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats (300–350 g body weight) were used in the present experiment. All animals were housed individually in a temperature (22°C) and light controlled (12 h light:dark cycle, lights on at 06.00 h) colony room. Food and water were available ad libitum. All treatments adhere to the NIH Guide for the Care and Treatment of Laboratory Animals and were approved by the Animal Care Committee at NEOUCOM.

2.2. General procedure

On the day of an experiment a male rat was sacrificed by rapid decapitation. The corpus striatum was dissected from both hemispheres. Following a midline bisection, the ventricles were pried opened and the corpus striatum was dissected out from within the perimeter of the corpus callosum. The corpus striatum was dissected into small tissue fragments ($0.5 \times 0.5 \times 0.5$ mm) prior to placement into the superfusion chamber. Following a 35–45 min equilibration period during which no samples were collected, effluent samples were collected at 10 min intervals for the duration of the 13 collection interval experiment. The superfusion medium consisted of a modified Kreb's Ringer phosphate (KRP) buffer: 123 mM NaCl, 4.8 mM KCl, 0.8 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.8 mM NaH_2PO_4, 10.2 mM Na_2HPO_4 and 0.18% glucose at pH 7.4. The KRP medium was filtered (Milipore Filter-0.45 μm) prior to use. The superfusion chamber consisted of the barrel of a 1 ml plastic tuberculin syringe cut off at the 0.3 ml level and was attached to a 22 gauge stainless steel syringe needle. This assembly was placed in a temperature controlled water bath that maintained the tissue at 37°C. The tissue fragments were suspended on cellulose filter paper within the chamber and were contained within 100 μl of medium volume. The chambers were sealed with a stopper containing two needles, one supplying filtered humidified air to the chamber to oxygenate the samples and the other serving as an exit port for collection of effluent samples. Superfusion medium was delivered through the bottom of the chambers at a flow rate of approximately 25 μl/min and effluent samples were collected at 10 min intervals into plastic tubes maintained on ice. At the end of the superfusion, tissue fragments were removed and weighed. The superfusion system for determination of in vitro catecholamine release has been extensively used and validated in our laboratory (Ramirez et al., 1985).

2.3. Assay

Assays for dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) concentrations were performed using high performance liquid chromatography (HPLC) with electrochemical detection (ESA). Biogenic amines were separated on a Biophase C-18, 5 μm sphere column (Bioanalytical Systems). The mobile phase consisted of 50 mM sodium acetate, 27.4 mM citric acid, 10 mM sodium hydroxide, 0.1 mM sodium octyl sulfate, 0.1 mM EDTA and 7% methanol in filtered distilled water. The final pH of 4.5 was obtained with the addition of sodium hydroxide and the mobile phase was filtered (Milipore filter-0.45 μm) prior to use. Standards for dopamine and DOPAC were diluted in the KRP superfusion medium and doses of 12.5, 25, 50, 100, 200 and 400 pg/20 μl were used to construct a standard curve. The sensitivity of this assay, as defined by a response reliably detectable over baseline noise, was $\leq$ 12.5 pg/20 μl.

2.4. Experiment 1

In experiment 1 the effects of a 10 min infusion of varying doses (0 (control), 0.1, 1, 5 and 10 μM) of nicotine (Sigma) upon both spontaneous as well as subsequent potassium chloride stimulated dopamine and DOPAC output from the corpus striatum were tested. Tissue fragments from an individual rat were divided among several superfusion chambers and subjected to the five doses of nicotine infusion. These experiments were replicated with separate rats until the following N values were achieved: 0, $N = 9$; 0.1, $N = 5$; 1, $N = 5$; 5, $N = 4$ and 10, $N = 7$. After a three interval basal collection period, nicotine,

diluted in the KRP superfusion medium was infused for a 10 min period at the start of collection interval 4. The normal KRP medium was resumed with the start of collection interval 5 and continued until the start of collection interval 10 when KRP medium containing 30 mM potassium chloride was infused for a 10 min interval. The sodium chloride concentration was adjusted during the infusion of the potassium chloride to maintain the osmolarity of the medium at 290 mOsm. With the start of collection interval 11, the normal KRP medium was resumed and continued until the end of the superfusion at collection interval 13. In this way, it was possible to evaluate the dose dependent effects of nicotine infusion upon both the spontaneous as well as the subsequent potassium chloride stimulated output of dopamine and DOPAC from the superfused corpus striatal tissue fragments.

2.5. Experiment 2

The results of experiment 1 showed that the two highest doses of nicotine (5 and 10 μM) completely abolished the subsequent potassium chloride stimulated output of dopamine. One possible explanation for such an effect was that the stimulated output from this high dose of nicotine depleted available dopamine stores for potassium chloride stimulation. To test this possibility, in experiment 2 the effect of an initial infusion of various dopamine secretagogues upon subsequent potassium chloride stimulated output of dopamine was examined. The basic procedure for experiment 2 was similar to that of experiment 1 with the exception that during collection interval 4 one of the following four agents were compared for their capacity to evoke dopamine output: (1) normal KRP medium-control ($N = 9$), (2) 30 mM potassium chloride ($N = 7$), (3) 10 μM nicotine ($N = 7$) or (4) 10 μM amphetamine ($N = 14$). For each of these four conditions the normal KRP medium was resumed with the start of collection interval 5 and continued until all preparations were challenged with an infusion of 30 mM potassium chloride during collection interval 10. As described for experiment 1, the normal KRP medium was resumed with the start of collection interval 11 and continued until the end of the superfusion at collection interval 13. The selection of these potassium chloride (30 mM) and amphetamine (10 μM) doses was based upon extensive data using our superfusion system which demonstrates a clear and reliable, but not exhaustive, output of dopamine from striatal tissue fragments. In addition, it has been proposed that the use of this relatively low dose of amphetamine displaces dopamine primarily from the cytoplasm through a Ca^{2+} independent process (Seiden and Sabol, 1993). Since the KRP controls along with the 10 μM nicotine infusion groups were run separately within the two experiments, these data were pooled and included within the analyses of both experiments 1 and 2.

2.6. Analyses

In order to analyze the release rate data, the intervals of the superfusion were blocked into three discrete segments: (1) basal release-collection intervals 2–4, (2) infusion evoked release-collection intervals 5–7 (8) and (3) potassium chloride release-collection intervals 11–13. Separate one-way analyses of variance (ANOVAs) were performed on each of these three periods of the superfusion. Pair-wise post-hoc comparisons were performed using the Fisher's LSD test. A $P < 0.05$ was required for results to be considered statistically significant.

3. Results

3.1. Experiment 1

In Fig. 1A are presented the DA release rate profiles for the five doses of nicotine tested. Summaries of the analyses of these data during the nicotine infusion (collection intervals 5–7) and potassium chloride (collection intervals 11–13) periods of the superfusion are contained within the bar graphs of Fig. 1B and C, respectively. Analyses of the basal dopamine release rates (collection intervals 2–4) revealed no overall statistically significant differences ($F(4, 25) = 1.79$, $P = 0.16$). For the nicotine infusion period of the superfusion (Fig. 1B), an overall statistically significant difference was obtained ($F(4, 25) = 11.63$, $P < 0.0001$). Post-hoc pairwise comparisons indicated that the amount of dopamine evoked by the 1, 5 and 10 μM infusion of nicotine was significantly greater ($P < 0.03$) than that of the 0.1 and 0 (control) μM nicotine doses. Maximal dopamine was evoked in response to the 5 μM nicotine dose and these levels were significantly greater than the 1 ($P < 0.01$), but not the 10 μM nicotine dose ($P = 0.08$). An overall statistically significant difference was obtained for the potassium chloride stimulated dopamine release rates ($F(4, 25) = 4.73$, $P < 0.006$) as summarized in Fig. 1C. As can be seen from the dopamine release rate profiles of Fig. 1A, the potassium chloride evoked output was completely abolished in preparations receiving the 5 and 10 μM nicotine infusion and the post-hoc comparisons confirm that these responses were significantly lower ($P < 0.05$) in the 5 and 10 versus the 0, 0.1 and 1 μM nicotine infusion groups. No significant differences were obtained between the 5 and 10 μM nicotine groups ($P = 0.09$), nor were any statistically significant pairwise differences in potassium chloride evoked release obtained among the 0, 0.1 and 1 μM nicotine groups ($P \geq 0.31$).

The corresponding DOPAC release rate profiles of experiment 1 are contained within Fig. 2A and summaries of the analyses for the nicotine infusion and potassium chloride stimulated periods are contained within Fig. 2B and C,

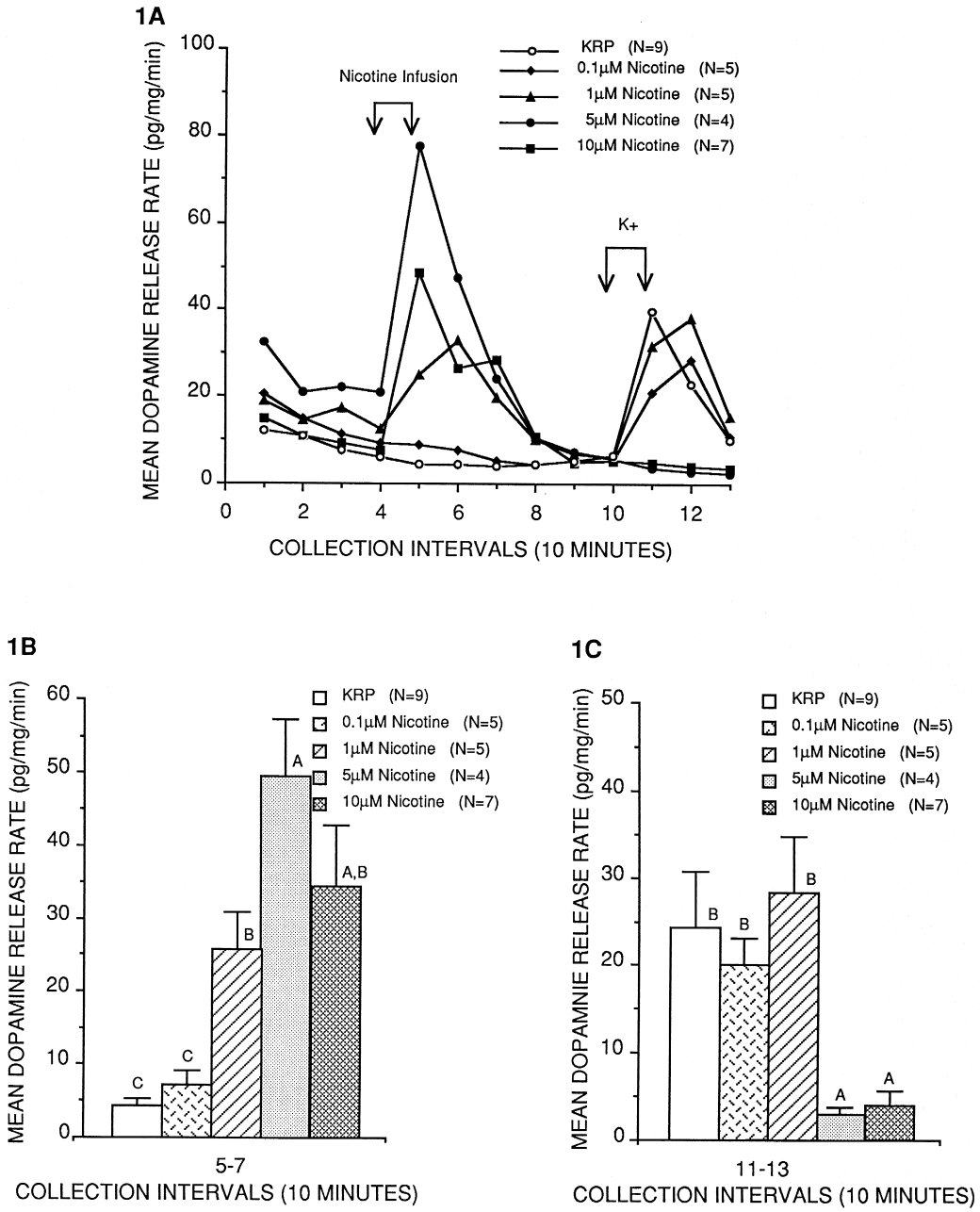

Fig. 1. (A) Dopamine release rate profiles (mean, pg/mg/min) from superfused corpus striatal tissue fragments of male rats. Following three collection intervals of spontaneous (basal) dopamine release, nicotine diluted in the Kreb's Ringer phosphate (KRP) superfusion medium was infused for a 10 min interval during collection interval four. Varying doses of nicotine (0 (KRP control), 0.1, 1, 5 and 10 μM) were tested in these preparations. All conditions were challenged with an infusion of potassium chloride (K^+, 30 mM) during collection interval ten of the superfusion. A summary of the analyses of these release rate responses during the periods of maximal responsiveness to nicotine and potassium chloride infusions is contained within (B) and (C), respectively. The S.E.M. bars are omitted from these release rate profiles for purposes of clarity, but are contained within the data analyses summary bar graphs. (B) Summary of dopamine release rates following nicotine infusion. Data represent responses obtained as calculated from collection intervals 5–7 of the superfusion (mean $\pm$ S.E.M., pg/mg/min). Dopamine evoked by the 1, 5 and 10 μM doses of nicotine were significantly greater than the 0.1 and 0 (control) μM nicotine doses ($P < 0.03$). Dopamine evoked by the 5 μM dose of nicotine was significantly greater than the 1 μM nicotine ($P < 0.01$). Bars with differing lettered notations indicate groups which are significantly different. (C) Summary of dopamine release rates following potassium chloride (30 mM) infusion. Data represent responses obtained as calculated from collection intervals 11–13 of the superfusion (mean $\pm$ S.E.M., pg/mg/min). Dopamine evoked by potassium chloride was significantly greater in groups previously infused with the 0 (control), 0.1 and 1 μM compared to the 5 and 10 μM nicotine groups ($p < 0.05$). Bars with differing lettered notations indicate groups which are significantly different.

respectively. There was no overall statistically significant difference in the basal output of DOPAC among the five treatments ($F(4, 25) = 0.94$, $P = 0.98$). An overall statistically significant difference was obtained for the nicotine infusion period of the superfusion ($F(4, 25) = 3.20$, $P < 0.03$) as summarized in Fig. 2B. Post-hoc comparisons

indicated that the DOPAC levels of the 1, 5 and 10 μM nicotine groups were significantly greater ($P < 0.03$) than that of the 0 μM (KRP control) group. For the potassium chloride stimulated response (Fig. 2C) there was no overall statistically significant difference among the five groups ($F(4, 25) = 2.02,\ P = 0.12$).

3.2. Experiment 2

The dopamine release rate profiles for the four groups included within experiment 2 are shown in Fig. 3A, while the corresponding data analyses summaries for the nicotine infusion and potassium chloride stimulated periods of the

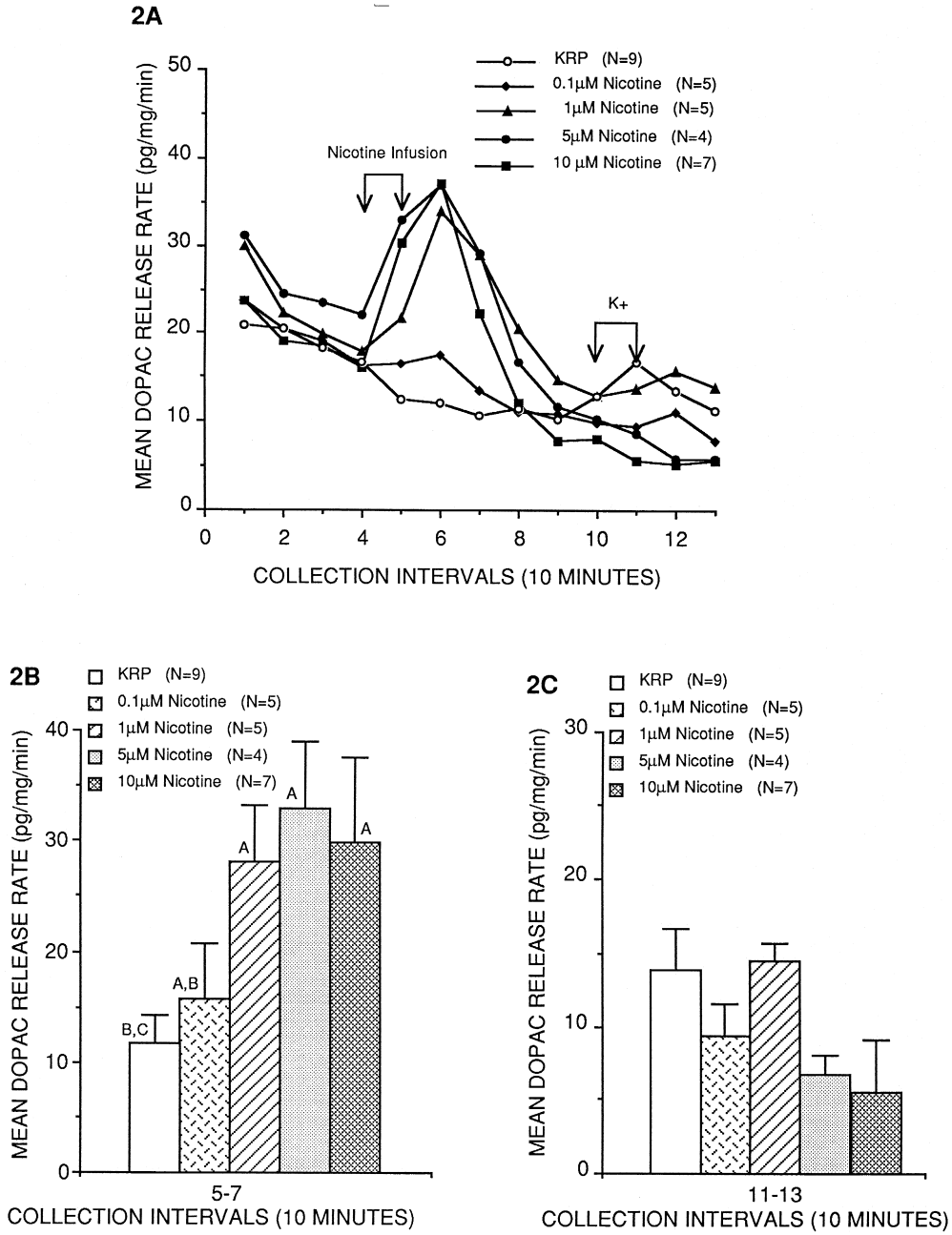

Fig. 2. (A) DOPAC release rate profiles (mean, pg/mg/min) from preparations as described in Fig. 1A. A summary of the release rate responses during the periods of maximal responsiveness to nicotine and subsequent potassium chloride infusions is contained within (B) and (C), respectively. (B) Summary of DOPAC release rates following nicotine infusion. Data represent responses obtained as calculated from collection intervals 5–7 of the superfusion (mean ± S.E.M., pg/mg/min). DOPAC levels evoked by the 1, 5 and 10 μM nicotine groups were significantly greater ($P < 0.03$) than that of the 0 (control) μM group. Bars with differing lettered notations indicate groups which are significantly different. (C) Summary of DOPAC release rates following potassium chloride (30 mM) infusion. Data represent responses obtained as calculated from collection intervals 11–13 of the superfusion (mean ± S.E.M., pg/mg/min). No overall statistically significant differences were obtained among the five groups.

superfusion are contained within Fig. 3B and C, respectively. Analyses of the basal dopamine release rates indicated an overall statistically significant difference among the four groups ($F(3, 33) = 3.13$, $P < 0.04$). Post-hoc analyses revealed that the basal release rates of the groups to be infused with the amphetamine were significantly

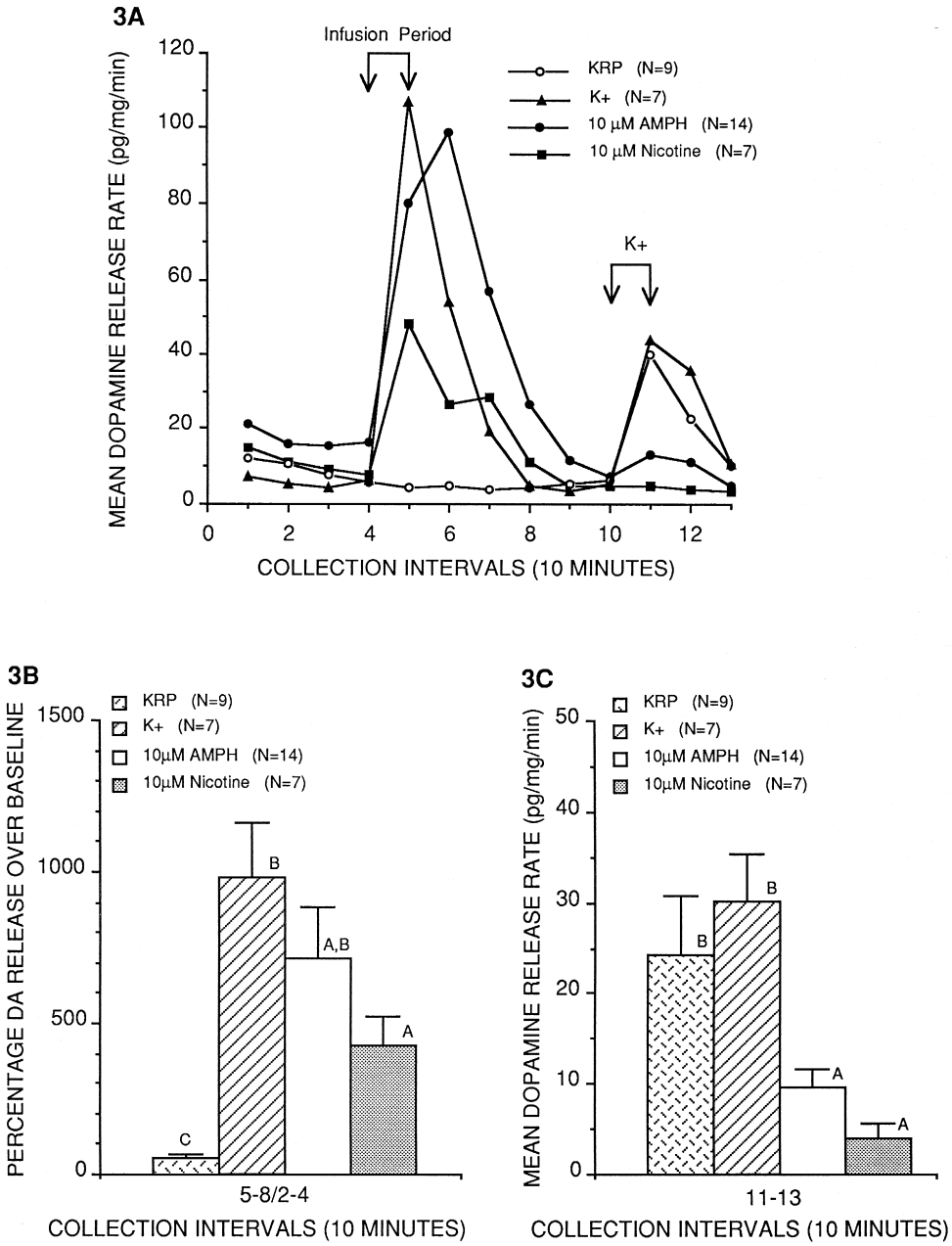

Fig. 3. (A) Dopamine release rate profiles (mean, pg/mg/min) from superfused corpus striatal tissue fragments of male rats. Following three collection intervals of spontaneous (basal) dopamine release, either nicotine (10 μM), amphetamine (AMPH, 10 μM), potassium chloride (30 mM) or Kreb's Ringer phosphate (KRP) superfusion medium was infused for a ten min interval during collection interval four. All conditions were subsequently infused with potassium chloride (K^+, 30 mM) during collection interval ten of the superfusion. A summary of the analyses of these release rate responses during the period of maximal responsiveness to these agents is contained within (B) and (C). The S.E.M. bars are omitted for purposes of clarity, but are contained within the data analyses summary bar graphs. (B) Summary of dopamine release rates following infusion of either 10 μM nicotine, 10 μM amphetamine (AMPH), 30 mM potassium chloride (K^+) or Kreb's Ringer phosphate (KRP) medium. Since there were variations in pre-infusion dopamine release rates, the data were normalized by evaluating responses following infusions of these agents as calculated from collection intervals 5–8 divided by their respective basal release rates as calculated from collection intervals 2–4. Dopamine evoked by potassium chloride was significantly greater than that evoked by nicotine ($P < 0.03$) or KRP medium ($P < 0.004$). Dopamine evoked by amphetamine or nicotine was significantly greater than that of the KRP control group ($P < 0.005$). Bars with differing lettered notations indicate groups which are significantly different. (C) Summary of dopamine release rates following potassium chloride (30 mM) infusion. Data represent responses obtained as calculated from collection intervals 11–13 of the superfusion (mean ± S.E.M., pg/mg/min). Dopamine evoked by potassium chloride in the potassium chloride (K^+) and KRP (control) groups was significantly greater than that of the nicotine and amphetamine groups ($P < 0.009$). Bars with differing lettered notations indicate groups which are significantly different.

greater than the groups to be infused with the potassium chloride ($P < 0.01$) and the KRP control ($P < 0.04$). Since this difference was present in the basal release rates among the groups prior to the infusion period, the dopamine release rate data of the infusion period was normalized for each superfusion chamber's basal release rate. This was accomplished by dividing the dopamine output during the period of maximal infusion-evoked responsiveness of the superfusion (5–8) by that of their respective outputs during the basal collection intervals (2–4). The results of this analysis showed an overall statistically significant difference ($F(3, 33) = 6.21$, $P < 0.002$) and are presented in Fig. 3B. Post-hoc comparisons of these adjusted release rate data indicate that the dopamine output evoked by the (first) potassium chloride infusion was significantly greater than that of the 10 μM nicotine ($P < 0.03$) and the KRP

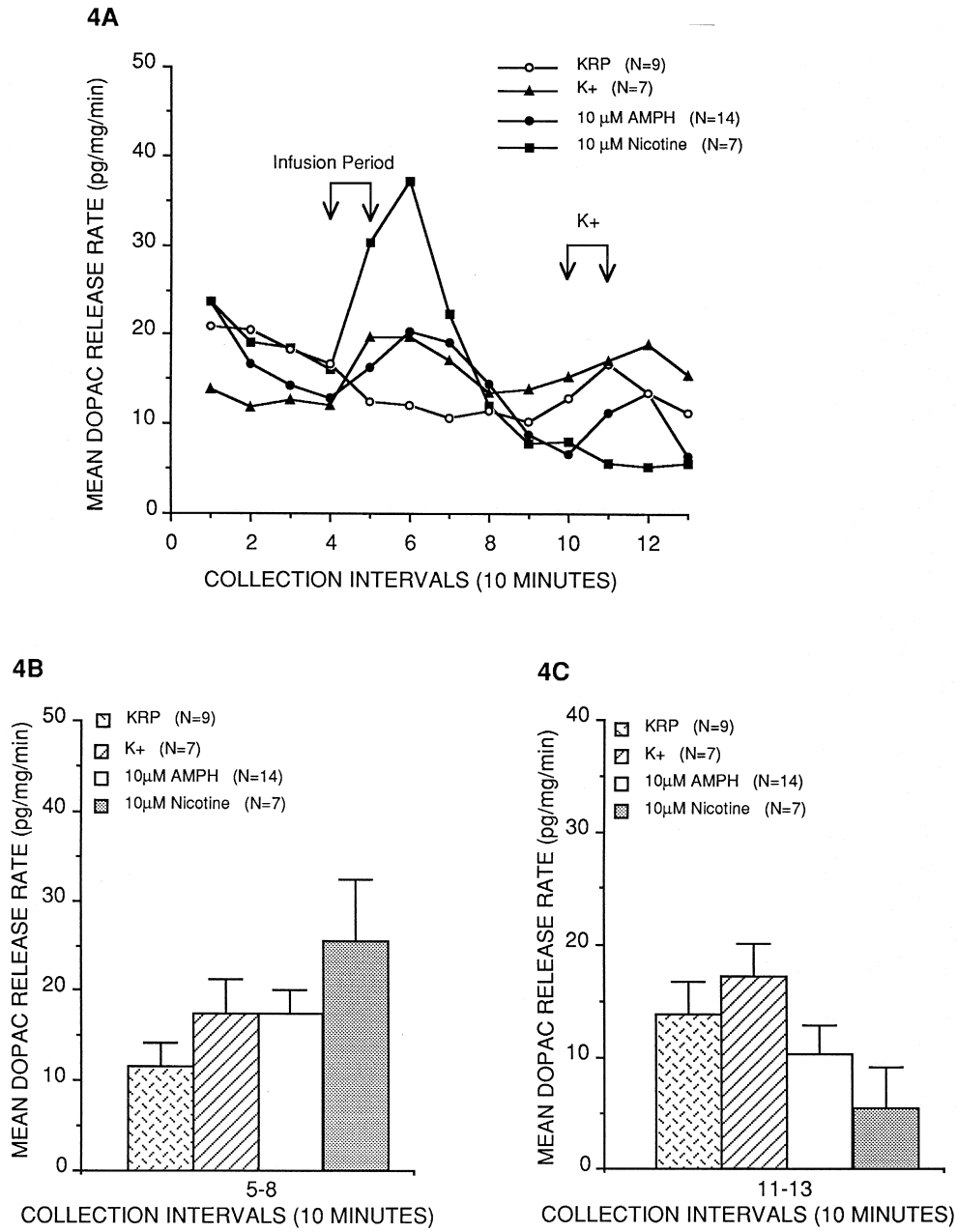

Fig. 4. (A) DOPAC release rate profiles (mean, pg/mg/min) from preparations as described in Fig. 3A. A summary of the release rate responses during the periods of maximal responsiveness to infusions of nicotine, amphetamine (AMPH), potassium chloride (K^+) and Kreb's Ringer phosphate (KRP) superfusion medium and responses to a subsequent infusion of potassium chloride are contained within (B) and (C), respectively. (B) Summary of DOPAC release rates following infusion with either 10 μM nicotine, 10 μM amphetamine (AMPH), 30 mM potassium chloride (K^+) or Kreb's Ringer phosphate (KRP) superfusion medium as described in Fig. 3B. No overall statistically significant differences were obtained among the four groups. (C) Summary of DOPAC release rates following potassium chloride (30 mM) infusion. Data represent responses obtained as calculated for collection intervals 11–13 of the superfusion (mean ± S.E.M., pg/mg/min). No overall statistically significant differences were obtained among the four groups.

control ($P < 0.004$). In addition, the amphetamine ($P < 0.002$) and nicotine ($P < 0.005$) evoked dopamine release rates were significantly greater than that of the KRP control group. Since all release rates returned to equivalent levels prior to the (second) potassium chloride infusion, it was not necessary to make any adjustments in these data for analyses. The potassium chloride stimulated output of dopamine (Fig. 3C) showed an overall statistically significant difference among the four groups ($F(3, 33) = 8.09$, $P < 0.0005$). Pairwise post-hoc analyses revealed that the potassium chloride evoked outputs of dopamine from the potassium chloride and KRP control groups were significantly greater than that of the 10 μM nicotine and 10 μM amphetamine groups ($P < 0.009$).

The corresponding DOPAC release rate profiles of experiment 2 are contained within Fig. 4A and the summaries of these analyses of the infusion and potassium chloride evoked DOPAC output periods are shown in Fig. 4B and C, respectively. No overall statistically significant differences were obtained for the basal DOPAC release rates ($F(3, 33) = 0.39$, $P = 0.76$). Similarly, no overall statistically significant differences among the four groups were obtained for the analyses of the infusion ($F(3, 33) = 1.90$, $P = 0.15$) or the potassium chloride ($F(3, 33) = 2.18$, $P = 0.11$) release rate periods of the superfusion.

4. Discussion

The dopamine and DOPAC release rate profiles obtained in response to nicotine were very unique and unlike anything observed from that of the other agents used to evoke dopamine output. This was particularly true of the 10 and 1 μM nicotine. With an infusion of 10 μM nicotine a clear increase in dopamine output was obtained, while the subsequent response to potassium chloride was completely abolished in these, as well as the 5 μM nicotine, preparations. This lack of a response to potassium chloride would not seem attributable to the initial amounts of dopamine released to the 10 μM nicotine since an infusion of 1 μM nicotine produced an amount of dopamine release that was not significantly different from the 10 μM nicotine, but maintained a normal potassium chloride stimulated response. Moreover, an initial infusion of potassium chloride at 30 mM can evoke significantly greater outputs of dopamine as compared to 10 μM nicotine, while permitting a clear second stimulated response to potassium chloride as was observed in experiment 2. In another report where it was demonstrated that repeated infusions of nicotine diminished subsequent responses it was also concluded that such effects were not due to a depletion of a releasable dopamine pool (Rapier et al., 1988).

Analogous to the present findings, it has been reported that in vivo nicotine administration diminishes dopamine turnover within the corpus striatum of both mice (Haikala et al., 1986) and rats (Fuxe et al., 1977). More related to the present conditions are data showing that repeated in vitro infusions of nicotine into striatal synaptosomal preparations produced reductions in dopamine release in response to a second or third infusion of nicotine (Rapier et al., 1988; Rowell and Hillerbrand, 1994). However, somewhat at odds with the present results are those data showing no change in either potassium chloride or amphetamine stimulated dopamine release when the preparations received a prior infusion of 5 μM nicotine (Rowell and Hillerbrand, 1994). Two salient differences between these and the present results are the doses of potassium chloride used (15 versus 30 mM, respectively) and the synaptosomal versus corpus striatal tissue fragment nature of the preparations. This latter factor may be particularly significant since it has been observed that clear differences in corpus striatal responses occur to nicotine as a function of whether synaptosomal or slice preparations are tested (Izenwasser et al., 1991; Marshall et al., 1996). While synaptosomal preparations may enable a better understanding regarding some of the mechanistic features of nerve terminal responses to nicotine, the relatively more intact integrity of the slice tissue preparation may provide a slightly better assessment of overall responses to nicotine.

A second notable feature of the 10 (as well as the 5 and 1) μM nicotine infusions were the high levels of DOPAC output evoked. Again, this was not observed in response to the potassium chloride or amphetamine stimulations. The simultaneous elicitation of high levels of dopamine and DOPAC suggest at least two potential mechanisms of action. One possibility may be that the high levels of dopamine released by nicotine can be rapidly taken up, metabolized to DOPAC and released from these nerve terminals. In this scenario, nicotine at doses ranging from 1–10 μM would be viewed as an agent which both evokes release and accelerates uptake. There is substantial evidence indicating that nicotine evokes striatal dopamine release (Izenwasser et al., 1991; Rowell and Hillerbrand, 1994; Whiteaker et al., 1995; Marshall et al., 1996; Dluzen and Anderson, 1997). While there is a report which indicates that nicotine can facilitate dopamine uptake from the mesolimbic system (Hart and Ksir, 1996), inhibition of dopamine uptake has also been observed (Izenwasser et al., 1991; Izenwasser and Cox, 1992). Our results tend to favor the suggestion that nicotine may be evoking release and inhibiting uptake. Such effects, stimulation of release and inhibition of uptake, resemble those obtained with amphetamine and the data of experiment 2 indicate some salient similarities between results obtained for 10 μM nicotine and amphetamine. Both show severely diminished potassium chloride stimulated dopamine responses, which differ significantly from the potassium chloride and KRP controls (Fig. 3A and C). Both show dopamine responses to their respective initial infusions that fail to differ significantly (Fig. 3B). Finally, both are characterized by dopamine release rate profiles which show an extended or

prolonged output as opposed to the more rapid rise and fall in dopamine output observed in response to potassium chloride (Fig. 1A). Interestingly, when the DOPAC release rate profiles are compared a clear distinction between nicotine and amphetamine is apparent. Whereas nicotine infusions result in high levels of DOPAC output, this does not occur in response to amphetamine. Therefore, in addition to evoking dopamine release and inhibiting dopamine uptake, our results suggest the additional possibility that nicotine increases intraneuronal metabolism of dopamine to DOPAC. There are data which support the concept that DOPAC is generated from intraneuronal stores of dopamine (Arbuthnott et al., 1990; Brannan et al., 1990; Xu and Dluzen, 1996). The present results suggest that nicotine may augment this process as indicated by the high levels of DOPAC observed in our superfusate samples following nicotine infusion. The appeal of this proposed tripartite mechanism (evoke release, inhibit uptake, increase intraneuronal dopamine to DOPAC metabolism) is its ability to explain the simultaneous increases in dopamine and DOPAC which would then effectively diminish stores of dopamine to abolish the second response to potassium chloride stimulation. However, considerably more work will be required to provide support for such speculation.

The results of experiment 1 show a dose related increase to the nicotine infusions. Such dose dependent responses have been reported previously (Rowell, 1995). Under the present conditions, overall dopamine responses were maximal to the 5 μM nicotine infusion (Fig. 1B). However, an inspection of the release rate profile from this group (Fig. 1A) indicates a slightly elevated basal/spontaneous efflux which may have contributed to the maximal levels obtained with this group. Analyses of the dopamine release evoked by nicotine showed clear increases in response to the 1, 5 and 10 μM nicotine with levels obtained from these groups being significantly greater than both the 0.1 and KRP controls. The accompanying DOPAC outputs revealed a slightly different profile as levels generated from the 1, 5 and 10 μM groups were significantly greater than the KRP controls, but not the 0.1 μM nicotine. In this way, the 0.1 μM nicotine dose is exerting differential effects upon dopamine and DOPAC that are not observed in response to the other nicotine doses. It has been reported that nicotine concentrations required to inhibit dopamine uptake are considerably lower than that needed to evoke dopamine output (Izenwasser et al., 1991). Therefore, one possible explanation for this differential between dopamine and DOPAC responses as a function of nicotine dose may be related to the actions upon these two mechanisms. Alternatively, DOPAC responses may not be sufficiently sensitive to discriminate clearly among these treatments. Since the range of differences among the groups for DOPAC responses is only in the order of two-fold while that for dopamine can be in the order of 10-fold, this possibility also deserves consideration.

Many different effects of nicotine upon nigrostriatal dopaminergic function have been reported. To account for these myriad of actions a variety of alterations upon dopamine mechanisms have been proposed. It has been suggested that nicotine can alter dopamine uptake (Izenwasser et al., 1991; Izenwasser and Cox, 1992), release (Rapier et al., 1988; Izenwasser et al., 1991; Rowell and Hillerbrand, 1994; Rowell, 1995; Sacaan et al., 1995; Marshall et al., 1996; Dluzen and Anderson, 1997) and metabolism (Haikala et al., 1986; Liekola-Pelho et al., 1990; Tsai and Lee, 1995). The present results support these findings of a complex array of actions of nicotine upon nigrostriatal dopaminergic function when assessed using superfused striatal tissue fragments. Like that observed by others the specific actions obtained appear critically dependent upon the dose of nicotine tested as well as the preparation used (synaptosomal versus slices). Our present data would support the conclusion of others that nicotine can evoke dopamine release and decrease dopamine uptake into striatal nerve terminals. With the addition of the DOPAC determinations our data expand upon these effects to suggest that nicotine may also be involved with increases in intraneuronal metabolism of dopamine to DOPAC. Such an action would be responsible for the increased levels of both dopamine and DOPAC in perfusate samples and effectively deplete dopamine stores within these neurons to render them unresponsive to a subsequent potassium chloride challenge.

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