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Intrategmental infusion of cocaine decreases dopamine release and enhances norepinephrine release in the medial prefrontal cortex

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Abstract

We evaluated the effects of local cocaine infusion into the A10 (ventral tegmental area), the cell body of the mesocorticolimbic dopaminergic pathway, on the extracellular concentrations of dopamine and norepinephrine in the medial prefrontal cortex, one of its terminal fields. A 1-ml Hamilton syringe was used to infuse a cocaine solution, either 20 or 200 μM, into the ventral tegmental area of anesthetized rats for 120 min through a microdialysis probe. The pure artificial cerebrospinal fluid (0 μM cocaine) infusion served as a control and a lidocaine (100 μM) infusion was administered to prevent the local anesthetic effect of cocaine. After intrategmental cocaine infusion (either 20 or 200 μM), extracellular dopamine and norepinephrine in the ventral tegmental area both increased significantly to a steady state level (208 ± 42 and 148 ± 23% for low dose and 220 ± 24 and 150 ± 15% for high dose). Simultaneously, the 200-μM cocaine infusion caused a significant decrease in extracellular dopamine (77 ± 5%) but an increase in norepinephrine (140 ± 6%) in the medial prefrontal cortex. The local anesthetic, lidocaine, produced no effects on the dopamine or norepinephrine output (neither in the ventral tegmental area nor in the medial prefrontal cortex). This study not only supports recent findings of an increase in extracellular dopamine and norepinephrine in the ventral tegmental area on intrategmental cocaine infusion, but also demonstrates that cocaine infused locally in the ventral tegmental area can decrease dopamine and increase norepinephrine at a remote terminal area (medial prefrontal cortex). Finally, the introduction rate of cocaine into the ventral tegmental area by retrograde microdialysis was found to be 0.83 ng/min for the low dose and 8.14 ng/min for the high dose.

Keywords: Microdialysis; Chloral hydrate; (Rat)

1. Introduction

The cell bodies of the mesocorticolimbic dopaminergic system are located in the ventral tegmental area (A10) and they project into the nucleus accumbens, olfactory tubercle, frontal cortex, amygdala, and septal area (Cooper et al., 1991; Weiner and Molinoff, 1994). This dopaminergic system has been recognized as one of the important sites for cocaine reinforcement. For example, (1) in 1982, Roberts and Koob (1982) reported that i.v. self-administration of cocaine is markedly disrupted following 6-hydroxydopamine lesioning of the ventral tegmental area. Also, (2) 6-hydroxydopamine lesions of nucleus accumbens reduce or eliminate i.v. self-administration of cocaine (Pettit et al., 1984; Roberts et al., 1980). Finally, (3) the intracranial self-administration of cocaine directly into the medial prefrontal cortex can be maintained by rats (Goeders and Smith, 1983, 1986).

Use of in vivo microdialysis and in vivo voltammetry has allowed several workers to demonstrate that both the extracellular dopamine in the ventral tegmental area (Bradberry and Roth, 1989; Broderick, 1992; Kalivas and Duffy, 1993; Moghaddam and Bunney, 1989) and in the medial prefrontal cortex (Maisonneuve et al., 1990; Moghaddam and Bunney, 1989; Luoh et al., 1994) increased after systemic cocaine injection. This is mainly because cocaine reaches both brain regions (Pan et al., 1994) and blocks the dopamine reuptake process (Heikkila et al., 1975). However, if cocaine reaches only the ventral tegmental area (e.g., microinjection), how will it affect the extracellular dopamine concentration in its terminal field (e.g., medial prefrontal cortex) in vivo: (1) by enhancing release, (2) by inhibiting release, or will it have no effect?

Electrophysicologists found, on the basis of intracellular recordings, that cocaine (1–10 μM) inhibited spontaneous firing and hyperpolarized the membrane of dopamine neu-

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rons in vitro (Brodie and Dunwiddie, 1990; Einhorn et al., 1988; Lacey et al., 1990; Pitts and Marwah, 1986) in the rat ventral tegmental area. They proposed the hypothesis that cocaine acts as a dopamine reuptake inhibitor in the ventral tegmental area, and that the resultant increase in extracellular dopamine acts upon dopamine autoreceptors to inhibit cellular activity. In 1994, Chen and Reith (1994a) used in vivo microdialysis and demonstrated that intrategmental cocaine infused into freely moving rats caused an increase in dopamine and norepinephrine in the ventral tegmental area, and that the increase was independent of cocaine's local anesthetic action. Later, they reported that dopamine and norepinephrine in the ventral tegmental area had complex interactions because (1) α_2-adrenoceptor antagonists could increase the dopamine and norepinephrine baseline in dialysates from the ventral tegmental area and (2) dopamine D_2 receptor antagonists could enhance the local, cocaine-induced, dopamine and norepinephrine increase in the ventral tegmental area (Chen and Reith, 1994b).

However, no one has yet studied the effects of cocaine infusion into the ventral tegmental area on extracellular dopamine and norepinephrine in its terminal field (medial prefrontal cortex), although it is known that the firing rate of dopamine neurons in vitro will decrease. Therefore, in this study, either 20 or 200 μM of cocaine solution was infused into the ventral tegmental area through a microdialysis probe (retrograde microdialysis). Rats with intrategmental infusion of artificial cerebrospinal fluid (aCSF; 0 μM cocaine) served as the control group. The effects on the extracellular dopamine and norepinephrine concentrations both in the ventral tegmental area and in the medial prefrontal cortex were then monitored and recorded. The dosage of cocaine introduced into the ventral tegmental area through a dialysis probe was also calculated from the data by measuring the cocaine concentrations in the perfusate, dialysate, and micropunched ventral tegmental area tissue.

2. Materials and methods

2.1. Animals and surgery

Male Sprague-Dawley rats (250–350 g weight; animal center, National Yang-Ming University, Taipei, Taiwan, ROC) were used. On the day of the experiment, the rats were anesthetized with chloral hydrate (400 mg/kg i.p.), then the right femoral vein was cannulated with a PE-50 polyethylene catheter (Clay-Adams, Parsippany, NJ, USA) for hourly supplying of anesthetics (80 mg/kg i.v.). The rats were fixed on a stereotaxic apparatus (Kopf models 1430 and 1460) and two microdialysis probes were inserted; one into the medial prefrontal cortex then, subsequently another into the ventral tegmental area. The coordinates used, from the bregma, were: AP, −5.8; L, ±2.9; V, −9.5 with an angle of 20° from the skull for the ventral tegmental area and AP, +3.1; L, ±0.8; V, −6.0 from the skull for the medial prefrontal cortex (Paxinos and Watson, 1982; Moghaddam and Bunney, 1989). The active membrane length of microdialysis probe was 1 mm for the ventral tegmental area and 4 mm for medial prefrontal cortex, respectively.

2.2. Catecholamine concentration assay section

After the first probe was inserted into the medial prefrontal cortex, it was perfused with aCSF (149 mM NaCl, 2.8 mM KCl, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 0.125 mM ascorbic acid and 5.4 mM D-glucose, pH 7.2–7.4) using a microliter syringe pump (Harvard model 55-3206) at a flow rate of 1.19 μl/min. After 2 h stabilization, we began collecting dialysate samples from the medial prefrontal cortex probe into 700-μl Eppendorf tubes at 20-min intervals. Samples (20 μl) were injected directly into either a dopamine or a norepinephrine high-performance liquid chromatography (HPLC) system for the separate

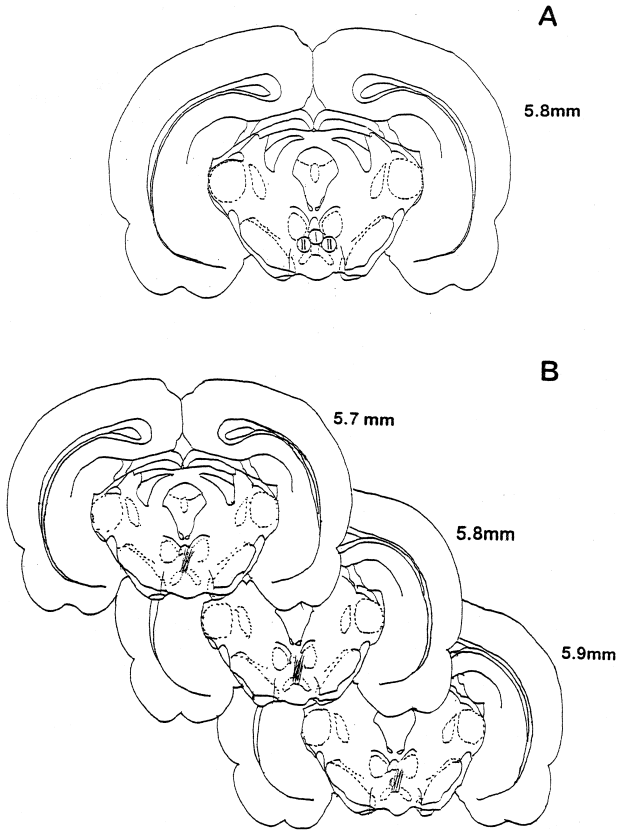

Fig. 1. (A) The micro-punched areas (zones 1 and 2) in rat ventral tegmental area. Zone 1 is the immediate site surrounding the infusion probe. Zone 2 is the region adjacent to the location of the infusion probe, where cocaine diffused along a concentration gradient. (B) Location of microdialysis probe membranes in the ventral tegmental area. These were recorded from 58 rat brain slices carefully selected during the histological verification. The drawing was made according to Paxinos and Watson (1986).

analysis of dopamine and norepinephrine. Due to the small amounts of sample obtained from each rat, the dopamine data and norepinephrine data were obtained from different rat groups. After obtaining stable baselines for dopamine or norepinephrine in the medial prefrontal cortex (needs approximately another 3 h), the second microdialysis probe was then implanted into the ventral tegmental area. After using the same procedure as with the first probe, not only were stable baselines of dopamine or norepinephrine in the ventral tegmental area obtained but also, the effects of the second probe in the ventral tegmental area on medial prefrontal cortex dialysate baseline levels of dopamine and norepinephrine were monitored. At this moment, the animals received a cocaine (0, 20, 200 μM in aCSF) or a lidocaine (100 μM in aCSF) infusion into the ventral tegmental area by retrograde microdialysis. After 120-min infusion, the perfusate containing cocaine was changed back to pure aCSF and then infused for another 60 min to further monitor extracellular dopamine and norepinephrine changes after termination of drug infusion.

2.3. Cocaine concentration assay section

In this section, the experimental procedure was the same as in the catecholamine concentration assay section, except that (1) dialysate samples collected from the infusion probe were injected into an HPLC with ultraviolet detector to analyze cocaine concentrations (but not dopamine and norepinephrine); and (2) at the conclusion of cocaine infusion, without further perfusion of aCSF for another 60 min, all rats were decapitated. The brain was quickly removed then frozen on dry ice. A frontal brain section (360 μm) was prepared with a cryostat, at 5.7 mm behind the bregma, then thaw-mounted onto glass slides. The ventral tegmental area (zones I and II; Fig. 1A) was dissected using a modified micropunch technique (Palkovits, 1973). Punched out tissues were placed into 40 μl mobile phase, which was to be used in a cocaine HPLC assay system and stored at −20°C until assayed. On the day of assay, tissue samples were thawed, sonicated, and centrifuged. A 20-μl portion of the supernatant was injected into the HPLC/UV system to quantify the content of cocaine. The tissue pellets were dissolved in 100 μl 1.0 M NaOH and assayed for their protein content (Lowry et al., 1951). The data were expressed as ng cocaine/μg protein.

2.4. Chromatography

The HPLC with electrochemical detector for dopamine determination consisted of a 100 × 2.1-mm ODS Hypersil C_{18} 5μ column (Hewlett-Packard, No. 799160D-552). The mobile phase (50 mM NaH_2PO_4, 2.6 mM octyl sulfate sodium salt, 0.27 mM EDTA, 5.0 mM triethylamine, 15% methanol, pH 5.7 with H_3PO_4) was delivered at 0.3 ml/min by an ISCO 260D pump. Dopamine was detected with a glassy carbon electrode maintained at 0.75 V rela-

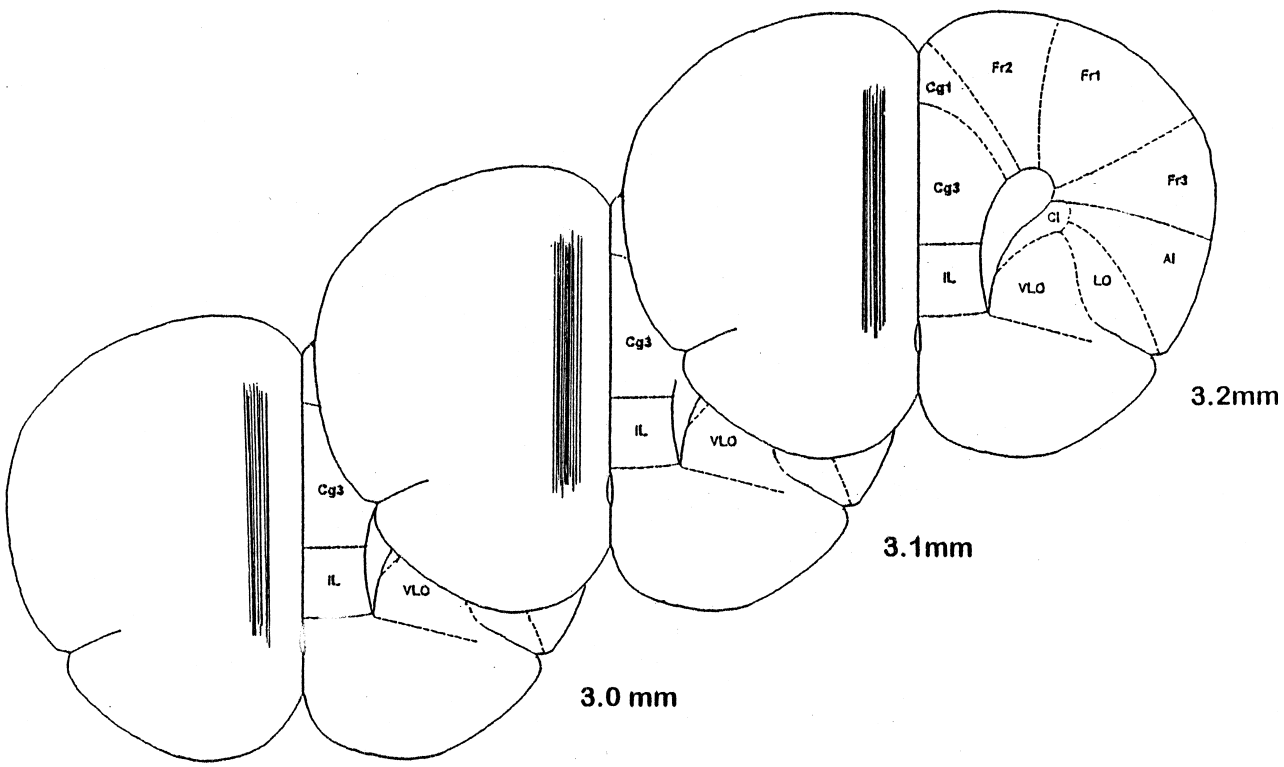

Fig. 2. Location of microdialysis probe membranes in the medial pre-frontal cortex. These were recorded from 58 rat brain slices carefully selected during the histological verification. The drawing was made according to Paxinos and Watson (1986).

tive to a Ag/AgCl reference electrode. The chart recorder was from Linear (model 1202). The dopamine system gave an on-column detection limit of 3.0 pg for dopamine.

The HPLC system for norepinephrine determination was similar to the dopamine system except that (1) the mobile phase (0.1 M sodium acetate, 0.1 mM EDTA-2Na, 2 mM 1-octanesulfonic acid, 8% methanol, pH 6.5 with H_3PO_4) was delivered at 0.25 ml/min and (2) the glassy carbon electrode was maintained at 0.65 V. The norepinephrine system gave an on-column detection limit of 3.4 pg for norepinephrine.

The HPLC/UV system for cocaine determination consisted of an ISCO 100D pump, LDC detector (model Spectromonitor 3200; wave length 235 nm) and Bioanalytical recorder (model RYYT-257). A self-packed 150 × 2.0-mm ODS Nucleosil C_{18} 5μ column was used and mobile phase (50 mM NaH_2PO_4, 5.0 mM triethylamine, 10% methanol, 17% acetonitrile, pH 5.6 with H_3PO_4) was delivered at a flow rate of 0.5 ml/min. The detection limit of cocaine was 6.1 ng.

2.5. Histology

Except for the rats in the cocaine concentration assay section, all animals were killed by an overdose of chloral hydrate at the end of the experiment. The brain was removed and fixed in 30% sucrose in a 10% formalin solution. Frozen 25-μm sections were stained with either Cresyl violet or Neutral red and were used for histological verification of the path of both probes.

2.6. Statistics

All values are presented as means ± S.E.M. A two-way analysis of variance (ANOVA) with repeated measurements was used to evaluate the difference between groups. This was followed by the post-hoc Scheffé multiple range test. All results were considered statistically significant at $P < 0.05$.

3. Results

A total of 68 rats were used. Six rats were withdrawn, either because some died midway in the experiment or the HPLC systems malfunstioned. Sixty-two rats were used for the entire experiment. After histological verification of the probe's path, 58 rats were included in our results. Figs. 1B–2 illustrate the locations of the dialysis probe in the ventral tegmental area and medial prefrontal cortex, respectively.

3.1. Effect of a second probe in the ventral tegmental area on medial prefrontal cortex dialysate baselines of dopamine and norepinephrine

As described in the experimental section, one microdialysis probe was first implanted into the medial prefrontal cortex. About 5 h after probe implantation, the dopamine or norepinephrine concentrations reached stable levels. These stable levels of dopamine or norepinephrine are the baseline dialysate concentration, which is different from the extracellular basal concentration. These baselines were 0.80 ± 0.13 nM for dopamine and 1.44 ± 0.20 nM for norepinephrine. The second probe was then implanted into the ventral tegmental area. During the equilibration of the second probe, the dialysate samples from the medial prefrontal cortex were being monitored continuously. The average values were calculated by the last three dialysate samples from the medial prefrontal cortex immediately before the baselines for dopamine and norepinephrine for the ventral tegmental area were obtained (1.23 ± 0.23 nM for dopamine and 1.59 ± 0.26 nM for norepinephrine). The average values were 0.84 ± 0.14 nM for dopamine and 1.34 ± 0.20 nM for norepinephrine. There was no significant difference between the baselines for dopamine and norepinephrine in the medial prefrontal cortex before and after probe implantation into the ventral tegmental area.

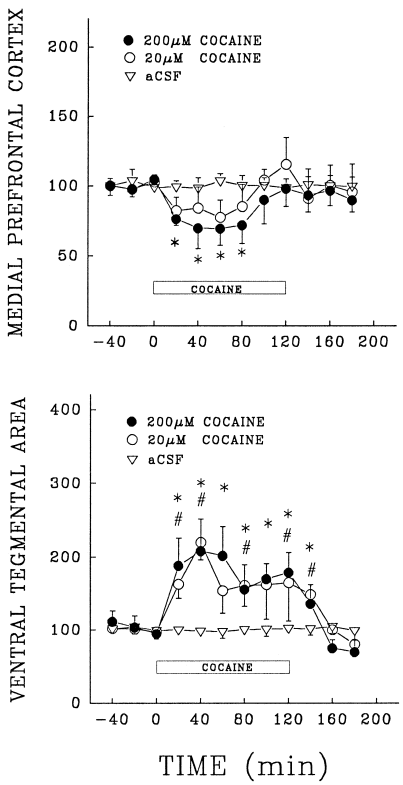

Fig. 3. Effects of intrategmental infusion of cocaine (either 20 or 200 μM) or aCSF via the microdialysis probe on extracellular dopamine in the ventral tegmental area (lower panel) and the medial pre-frontal cortex (upper panel). Values are percentile increases of baseline levels which were calculated as the averages of the last three time points in the pre-drug period. The time points are mean ± S.E.M. ($n = 6–7$ for each group). Square at the bottom indicates the period of cocaine infusion. Significant differences are indicated between the 200-μM group and the aCSF group (* $P < 0.05$) as well as between the 20-μM group and the aCSF group ($^{\#}$ $P < 0.05$) at comparable time points.

3.2. Catecholamine concentration

The changes in dialysate dopamine after intrategmental infusion of 0, 20, and 200 μM cocaine are shown in Fig. 3 for both ventral tegmental area (lower panel) and medial prefrontal cortex (upper panel). After 20-μM cocaine infusion, dopamine in the dialysate from the ventral tegmental area increased to a significant level at all time points ($F(32,99) = 5.52$, $P < 0.0001$; $208 \pm 42\%$ of baseline at maximum). Meanwhile, dopamine in the dialysate from the medial prefrontal cortex had a tendency to decrease but not significantly compared with that for the control group (0 μM cocaine infusion; $F(34,121) = 1.78$, $P < 0.01$; N.S.). After 200-μM cocaine infusion, the dopamine in the dialysate from the ventral tegmental area also increased significantly ($F(32,99) = 8.09$, $P < 0.0001$; $220 \pm 24\%$ baseline at maximum). in contrast to dopamine in the ventral tegmental area dialysate, dialysate dopamine from the medial prefrontal cortex decreased significantly ($F(33,110) = 2.27$, $P < 0.0008$; $77 \pm 5\%$). The medial prefrontal cortex dopamine decrease matched the ventral tegmental area dopamine increase at each of the comparable time points before the 80-min infusion. After the 80-min infusion, the decrease in dopamine in the medial prefrontal cortex disappeared.

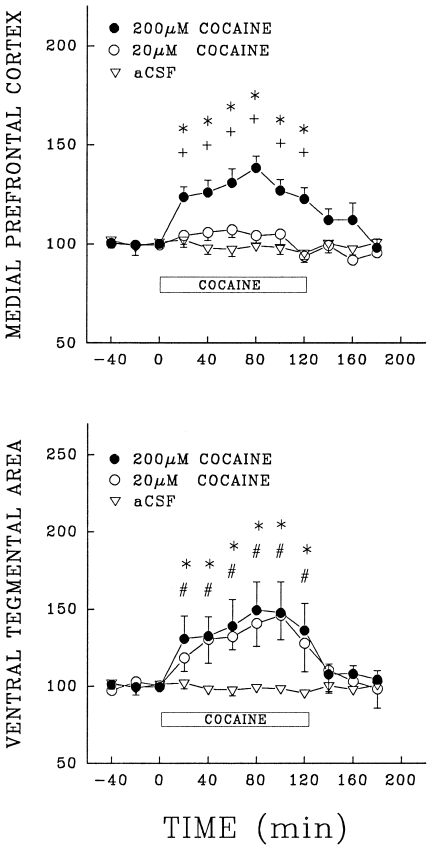

Fig. 4. Effects of intrategmental infusion of cocaine (either 20 or 200 μM) or aCSF via the microdialysis probe on extracellular norepinephrine the ventral tegmental area (lower panel) and the medial pre-frontal cortex (upper panel). Values are percentile increases of baseline levels which were calculated as the averages of the last three time points in the pre-drug period. The time points are mean ± S.E.M. ($n = 6$–10 for each group). Square at the bottom indicates the period of cocaine infusion. Significant differences are indicated between the 200-μM group and the aCSF group (* $P < 0.05$) as well as between the 20-μM group and the aCSF group ($^{\#}$ $P < 0.05$) at comparable time points. $^{+}$ $P < 0.05$ between the 200-μM group and 20-μM group is also indicated.

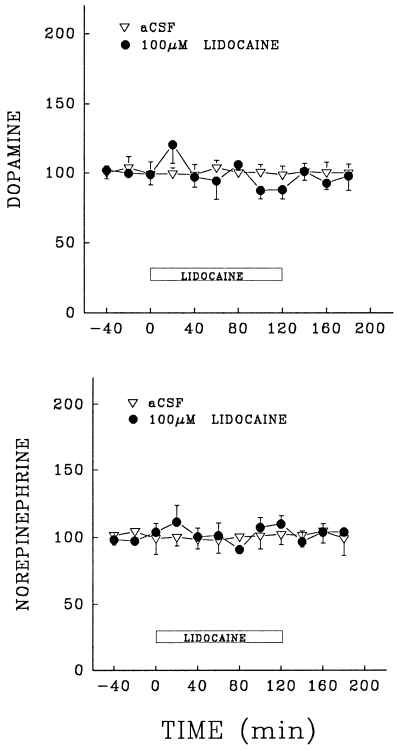

Fig. 5. Effects of intrategmental infusion of lidocaine (100 μM) via the microdialysis probe on extracellular dopamine (upper panel) and norepinephrine (lower panel) in the ventral tegmental area. Values are percentile increases of baseline levels which were calculated as the averages of the last three time points in the pre-drug period. The time points are mean ± S.E.M. ($n = 4$–6 for each group). Square at the bottom indicates the period of lidocaine infusion.

Fig. 4 shows that dialysate norepinephrine changed for both the ventral tegmental area (lower panel) and medial prefrontal cortex (upper panel) after cocaine infusion. After 20-μM cocaine infusion, dialysate norepinephrine from the ventral tegmental area increased significantly at all time points ($F(33,110) = 5.32$, $P < 0.0001$; $148 \pm 23\%$ of baseline at maximum). Meanwhile, dialysate norepinephrine from the medial prefrontal cortex also increased slightly but was not significantly different from that of the control group ($F(33,110) = 1.58$, $P < 0.04$; N.S.). After 200-μM cocaine infusion, dialysate norepinephrine from the ventral tegmental area also increased significantly ($F(37,154) = 9.49$, $P < 0.0001$; $150 \pm 15\%$ of baseline at maximum), then simultaneously, dialysate norepinephrine from the medial prefrontal cortex increased significantly at each comparable time point ($F(37,154) = 5.0$, $P < 0.0001$; $140 \pm 6\%$ of baseline at maximum). After termination of

cocaine infusion, both dopamine and norepinephrine increases in the ventral tegmental area returned to their baselines quickly.

Intrategmental lidocaine infusion caused no significant changes in extracellular dopamine and norepinephrine in either the ventral tegmental area (Fig. 5) or the medial prefrontal cortex (Fig. 6).

3.3. Cocaine concentration assay section

The changes of cocaine concentration in the ventral tegmental area dialysate over time during and after intrategmental cocaine infusion are shown in Fig. 7. After 20-μM cocaine infusion, the dialysate cocaine increased and reached steady state within 40 min (17.6 ± 0.5 μM). For the 200-μM cocaine infusion, the dialysate cocaine reached a steady state within 60 min (176.0 ± 3.5 μM). After termination of the cocaine infusion, dialysate cocaine decreased markedly during the first 20-min period but thereafter decreased slowly to its lowest level during the second 20-min period. Using the law of mass conservation, the introduction rate of cocaine is equal to [cocaine concentration in perfusate − cocaine concentration in dialysate] × microdialysis flow rate. This calculation yields introduction rates: 0.83 ng/min for 20 μM cocaine infusion and 8.14 ng/min for 200 μM cocaine infusion, respectively.

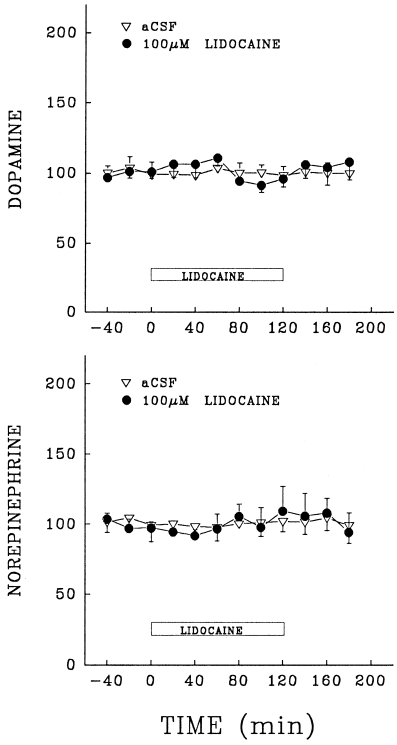

Fig. 6. Effects of intrategmental infusion of lidocaine (100 μM) via the microdialysis probe on extracellular dopamine (upper panel) and norepinephrine (lower panel) in the medial pre-frontal cortex. Values are percentile increases of baseline levels which were calculated as the averages of the last three time points in the pre-drug period. The time points are mean ± S.E.M. ($n = 4$–6 for each group). Square at the bottom indicates the period of lidocaine infusion.

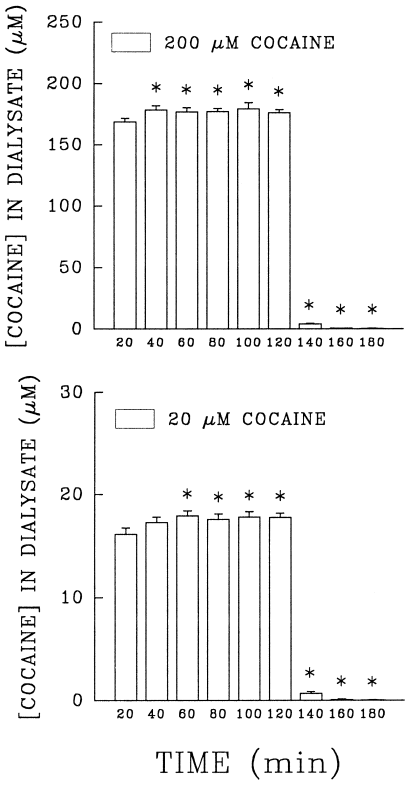

Fig. 7. Dialysate cocaine concentration from the intrategmental probe which was perfused with either 20 μM cocaine (lower panel) or 200 μM cocaine (upper panel) in aCSF. Samples were collected at 20-min intervals during the 120-min infusion period and after termination of cocaine infusion. Values are mean ± S.E.M. ($n = 6$ animals/group).

Cocaine was undetectable in the micropunched ventral tegmental area tissue; the cocaine content in ventral tegmental area tissue was therefore lower than 1.38 ng/μg protein. This indicates that introducing cocaine into the ventral tegmental area by reverse microdialysis results in the presence of cocaine around the probe's immediate embedded area, but not in the more distant adjacent tissue.

4. Discussion

This study yielded several important findings: (1) support for the previous finding of an increase in extracellular dopamine and norepinephrine in the ventral tegmental area on intrategmental cocaine infusion (Chen and Reith, 1994a,b); (2) the demonstration that intrategmental cocaine can decrease extracellular dopamine in the medial prefrontal cortex, which is consistent with the electrophysiological evidence of a decreased firing rate of dopamine neurons in vitro (Brodie and Dunwiddie, 1990; Lacey et al., 1990); also, (3) the demonstration that intrategmental cocaine can cause an increase of extracellular norepinephrine in the medial prefrontal cortex. These three findings

expand our viewpoint about the cause and the magnitude of the extracellular dopamine and norepinephrine increase in the medial prefrontal cortex after systemic cocaine injection (Pan et al., 1995). We must now ask whether; this is a direct terminal action or, is combined with an indirect influence from cell bodies? Further, there are reports that repeated intrategmental injection of central nervous system (CNS) stimulants can produce a sensitized locomotor response to subsequent injection of CNS stimulants (Kalivas and Weber, 1988; Vezina and Stewart, 1990). In order to understand the mechanism by which intrategmental injection of CNS stimulants causes sensitization, the effects of intrategmental injection of CNS stimulants on the cortical terminal field and other brain regions, need more exploration.

A major pharmacological property of cocaine is inhibition of the reuptake of dopamine, norepinephrine, and 5-hydroxytryptamine (Reith et al., 1986; Ritz et al., 1990). Predictably, cocaine locally applied into the ventral tegmental area increases dopamine, norepinephrine, and 5-hydroxytryptamine concentrations simultaneously. Although 5-hydroxytryptamine concentrations were not monitored in the present study, an increased 5-hydroxytryptamine concentration in the ventral tegmental area after locally applied cocaine has been reported elsewhere (Chen and Reith, 1994b). Increased dopamine in the ventral tegmental area exerts an inhibitory effect on ventral tegmental area dopamine cell firing through the D_2 dopamine receptor (Brodie and Dunwiddie, 1990; Lacey et al., 1990). Increased norepinephrine in the ventral tegmental area exerts an excitatory effect on the ventral tegmental area dopamine cell firing through α-adrenoceptors (Lategan et al., 1990, 1992). Also, increased 5-hydroxytryptamine exerts an excitatory effect on the ventral tegmental area dopamine cell firing by attenuating the γ-aminobutyric acid (GABA)-mediated feedback inhibition (Cameron and Williams, 1994). All these actions are thus involved in the overall effect of a decreased dopamine concentration in the medial prefrontal cortex. A possible explanation is that the percentile increase of the dopamine in the ventral tegmental area is greater than that of norepinephrine (220 vs. 140%) and the GABA-mediated feedback inhibition was not activated by locally applied cocaine. Therefore, the inhibitory effect of the dopamine D_2 receptor on ventral tegmental area dopamine neurons was stronger than the other effects.

In contrast to the dopamine decrease, the reason for norepinephrine increase in the medial prefrontal cortex after intrategmental cocaine infusion has not yet been explained. One of the possibilities is that intrategmental cocaine infusion might activate the ventral tegmental area-locus ceruleus projections (Deutch et al., 1986), thus causing the norepinephrine increase in its terminal field, the medial prefrontal cortex (Swanson and Hartman, 1975; Morrison et al., 1981). Another possibility is that the dopamine decrease in the medial prefrontal cortex could increase extracellular norepinephrine locally through an interaction between these two catecholaminergic systems (Tassin, 1992). More studies must be conducted to clarify this effect.

One may acknowledge that cocaine is a potent local anesthetic which reduces or eliminates impulse flow. Thus, infusion of cocaine into the cell body area (ventral tegmental area) reduces the terminal release of dopamine in the medial prefrontal cortex as expected and probably has little to do with complex circuitry or uptake blocking effects of cocaine. This was not likely to have been the case in our study because; (1) if cocaine can generally attenuate all neurons' activity through its local anesthetic property, it should decrease rather than increase the norepinephrine concentration in the medial prefrontal cortex; (2) intrategmental cocaine infusion has been reported to produce extracellular dopamine and norepinephrine increases in the ventral tegmental area and these effects have been proved to be mainly due to cocaine's property as a reuptake blocker, and not a result of its local anesthetic property (Chen and Reith, 1994a,b); and (3) local infusion of lidocaine (100 μM) through the microdialysis probe did not yield any changes of dopamine and norepinephrine concentrations in the medial prefrontal cortex in our control study (cf., Fig. 6A,B).

Currently, two popular techniques are used to apply chemicals locally into a specific brain region in vivo: (1) microinjection and, (2) reverse microdialysis. While microinjection offers some valuable results, it fails to provide any information about the drug concentration in the injection site area and also fails to circumvent pharmacokinetic factors. As demonstrated in the present study, microdialysis not only provides a clear figure of the drug's rate of introduction but also maintains a steady state cocaine level to circumvent pharmacokinetic factors. However, some of our colleagues expressed the fear that cocaine might diffuse into non-ventral tegmental area structures and possibly indirectly influence the terminal monoamine concentration during a prolonged period of infusion. The fact that, in our experiments, cocaine was undetectable in the micropunched ventral tegmental area tissue should allay these fears.

Intrategmental area administration of 200 μM cocaine via the dialysis probe produced dopamine and norepinephrine increases in the ventral tegmental area similar to those produced by intrategmental 20 μM cocaine. However, only intrategmental administration of 200 μM cocaine caused a significant decrease in dopamine and increase in norepinephrine in the medial prefrontal cortex. One explanation for these divergent effects on extracellular dopamine and norepinephrine in the medial prefrontal cortex may be diffusion of cocaine within the ventral tegmental area. Low-dose infusion has a lower concentration gradient of cocaine from the perfusion medium in the dialysis probe into adjacent brain tissue (small diffusion area) and the cocaine concentration in the area immediately adjacent to

the probe was sufficient to produce a plateau of the dopamine and norepinephrine increases in the ventral tegmental area. High-dose cocaine infusion will generate a larger diffusion area, thus a greater number of neurons in the somatodendritic area would be affected by cocaine. Therefore, the higher dose would cause a larger portion of the mesocortical dopamine presentation. Another possibility is that, since cocaine increases not only dopamine and norepinephrine but also 5-hydroxytryptamine, these three monoamine systems have very complex interactions (Chen and Reith, 1994b). The dissociation of the catecholamine response in the ventral tegmental area and that in the medial prefrontal cortex may come from interactions between all three monoamines.

In summary, the present results indicate that locally applied cocaine can decrease extracellular dopamine while simultaneously increasing extracellular norepinephrine in the medial prefrontal cortex. Since many input neurons from different brain regions project into the ventral tegmental area plus, many other local inter-neurons exist in the ventral tegmental area, the finding is that cortical terminal dopamine and norepinephrine changes must have a combined effect, therefore, the detailed mechanism of this phenomenon should be somewhat complicated. However, an understanding of the intrategmental cocaine effects on catecholamine in the medial prefrontal cortex may provide a lead.

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