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Effects of intravenous cocaine administration on cerebellar Purkinje cell activity

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Abstract

The goal of the present study was to investigate the effects of intravenous cocaine administration on cerebellar Purkinje cell firing. Extracellular neuron activity was recorded and cells were locally excited with spaced microiontophoretic pulses of glutamate. Glutamate-evoked and spontaneous discharges were compared before and immediately following cocaine administration. Cocaine injections (1.0 and 0.25 mg/kg, i.v.) induced a reversible suppression of both spontaneous activity and glutamate-evoked excitation. Procaine was ineffective in producing similar actions. Cocaine only inhibited glutamate-induced excitation in animals pre-treated with reserpine (5 mg/kg, i.p.). Propranolol injections (10 mg/kg, i.p.) were ineffective in blocking cocaine-induced inhibitions. Yohimbine (5 mg/kg, i.p.) pre-treatment abolished cocaine-induced suppressions of either spontaneous or glutamate-evoked excitation. Therefore, cocaine administration decreases Purkinje cell spontaneous and glutamate-evoked discharges by a mechanism involving α_2-adrenoceptor activation. It is suggested that by changing the normal function of the cerebellum cocaine can produce drug-related alterations in overt behavior and cognition.

Keywords: Cocaine; Cerebellum; Norepinephrine; Purkinje cell; (Rat); Microiontophoresis; Glutamate

1. Introduction

Cocaine is a potent local anesthetic agent, which is also well known for its alerting and psychomotor stimulant properties. Its major biochemical action is the inhibition of re-uptake mechanisms for all major monoamine transmitters including dopamine, 5-hydroxytryptamine (5-HT) and norepinephrine (Ritz et al., 1990). Cocaine's abuse liability derives from the fact that it activates endogenous "reward" circuits in the basal forebrain and, thus, serves as a powerful behavioral reinforcer (Gawin, 1991).

Although cocaine-rewarding effects have been attributed mainly to an action at dopaminergic synapses other monoaminergic systems may play an important role in the drug's psychostimulant and behavioral reinforcing actions. For example, recent evidence casts doubts about dopamine's singular role in producing pleasurable or rewarding sensations. Several studies have suggested that the output of dopamine-containing cells serves to identify reward-predicting cues (Spanagel and Weiss, 1999; Schultz et al., 1997; Bowman et al., 1996). Others have shown that dopamine cell activity is not directly related to the reward value of a stimulus but does highlight novel or startling events that re-direct attention to specific stimuli within the environment (Horvitz et al., 1997). Furthermore, it is well established that dopamine-transporter knockout mice still self-administered cocaine (Rocha et al., 1998). These studies direct our attention to the possible contribution of the other two catecholamines, 5-HT and norepinephrine, and their respective anatomical targets in the production of cocaine psychostimulant effects.

Information is emerging concerning the anatomical and physiological substrates underlying the facilitating effects of cocaine on sensory perception and other cognitive abilities. Studies from our laboratories (Jimenez-Rivera and Waterhouse, 1991; Waterhouse et al., 1996 and Bekavac and Waterhouse, 1995) have shown that parenterally and locally administered cocaine can enhance somatosensory cortical neuronal responsiveness to afferent excitatory synaptic inputs and iontophoretically applied glutamate. These actions mimicked previously demonstrated facilitat-

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ing effects of norepinephrine on neuronal responsiveness in cerebral cortex (Waterhouse et al., 1981; Woodward et al., 1991a; Mouradian et al., 1991) and elsewhere (Rogawski and Aghajanian, 1980). Recently, 5-HT has been implicated in the facilitating action of cocaine in cortex (Waterhouse et al., 1996).

In vivo experiments in rat cerebellum have shown that systemically or locally administered cocaine can mimic norepinephrine-induced augmentation of gamma-aminobutyric acid (GABA)-mediated suppression of Purkinje cell discharge (Waterhouse et al., 1991). Although, new data concerning cocaine's actions on motor related structures and its locomotor activating effects (Peoples et al., 1998; Hiroi et al., 1999; Fleckenstein et al., 1996; Peris, 1996) has been published, studies regarding cocaine's actions in the cerebellum, an area crucial to the control of locomotion, are lacking.

Recent research on the cerebellum demonstrates that this structure is more complex in terms of its physiology than what had been previously envisioned. The classical view of the cerebellum presented it as having a principal role in motor coordination. However, this notion has been expanded to include the cerebellum as having a significant part in motor learning, non-motor associative learning and cognitive functions (Thompson et al., 1998; Parsons et al., 1997; Drepper et al., 1999). Therefore, it is important to determine the cellular effects of cocaine in a structure such as the cerebellum for the implications it may have on brain function. Furthermore, it is well known that the excitatory neurotransmitter glutamate plays a crucial role in the development as well as the normal function and plasticity of the cerebellum (Burgoyne et al., 1993; Anwyl, 1999). Thus, the present investigation was directed at studying the effects of intravenous cocaine administration on spontaneous firing and glutamate-induced activation of cerebellar Purkinje neurons. Since some of cocaine's electrophysiological effects are mediated by norepinephrine and because it is known that the cerebellum receives a robust noradrenergic functional innervation (Woodward et al., 1991b), we also studied the possible involvement of norepinephrine in these actions. The results indicate that intravenous cocaine injections diminished Purkinje cell spontaneous activity and glutamate-induced activation via an α_2-adrenoceptor mediated mechanism. A preliminary report of some of these findings has appeared elsewhere (Jiménez-Rivera et al., 1994).

2. Methods

2.1. Animals and surgery

Male Sprague–Dawley rats from our colony (weight 275–320 g), were used in this study. Animals were initially anesthetized with chloral hydrate 400 mg/kg, i.p., intubated and allowed to breath spontaneously. Additional anesthesia was administered (chloral hydrate 100 mg/kg, i.p.) as needed. After initial anesthesia, animals were mounted in a stereotaxic apparatus and the skull and dura overlying the posterior vermis of the cerebellum were removed. The exposed brain was covered with Earl's balanced saline solution (Gibco, NY). All rats were cannulated with a femoral vein catheter (PE 60, Intramedic, NJ) for intravenous drug injections. Body temperature was maintained at 37°C with a DC heating pad (Frederick Haer, ME). All animal experimentation was carried out in accordance with the United States Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals. The procedures were approved by the Universidad Central del Caribe Institutional Animal Care and Use Committee.

2.2. Microiontophoretic and systemic drug administration

Five-barrel micropipettes, tip diameter 4–6 μm, were used to record spontaneous 'simple' spike discharge from individual Purkinje neurons (Eccles et al., 1967) and to apply chemical substances at the recording site by microiontophoresis. The center barrel filled with 3 M NaCl, was used for recording (3–6 MΩ impedance) single unit activity. Automatic current balancing was maintained via a side barrel filled with 3 M NaCl. The remaining barrels were filled with 1 M L-glutamate, pH 8.4 (Sigma). Neuronal activity was amplified (Dam80 amplifier, WPI) and input to a window discriminator (Brainwave, Datawave Technology, CO). The output of the discriminator was sent to a digital computer for on-line generation and display of continuous ratemeter records. Ratemeter records and raw spike train data were also stored for off-line analysis. Regularly spaced iontophoretic pulses of glutamate (1–50 nA, 10-s duration every 40 s) were applied to individual Purkinje neurons via a commercially available microiontophoresis machine (BH-2; Medical Systems, Great Neck, NY). Retaining currents (+10 nA) were applied routinely to drug barrels to prevent leakage of drug solutions into the surrounding tissue.

2.3. Parenteral drug administration

Intravenous injections of cocaine or procaine (doses of 0.25 or 1 mg/kg; Sigma, St. Louis, MO) were administered once a stable firing pattern was observed from a single neuron for a minimum of 5 min. Each drug injection was preceded by an isovolumetric equivalent solution of NaCl (0.9%) to determine control responses to the injection procedure. In pharmacologic studies, animals were injected with yohimbine (5 mg/kg, i.p.) and propranolol (10 mg/kg, i.p., Sigma) 10–15 min before cocaine administration. In reserpine-treated animals, reserpine (5 mg/kg, i.p., Sigma) was administered 5 h before the cocaine injection. All injectable compounds were dissolved and administered as a saline (0.9%) solution.

2.4. Data analysis

Glutamate-evoked and spontaneous discharge of individual cells were quantified by averaging the discharge rate of a cell during at least three transmitter applications for glutamate responses and by averaging the mean firing rate between drug pulses for spontaneous activity. For each cell, glutamate-evoked and spontaneous discharges were compared for a period of 200-s before and 200-s immediately following cocaine administration. This time epoch was chosen for analysis since in all cells tested cocaine actions were readily seen during this period. The time at cocaine's maximum effect was not employed in the analysis because at this time a large group of neurons had very low spontaneous activity, which would have been a confounding variable. All data were statistically analyzed with paired-sample student *t*-test (two-tailed) using a significance level of $P < 0.05$.

The following operational definitions were employed as in previous reports (Jimenez-Rivera and Waterhouse, 1991; Waterhouse and Woodward, 1980; Waterhouse et al., 1981) to assess the influence of cocaine on glutamate-induced excitation. "Enhancement" of the evoked response relative to background was declared when spontaneous firing was suppressed at least 15% more by cocaine application than was activity evoked by glutamate administration. "Potentiation" of evoked excitation was defined as an absolute increase in glutamate-induced response over control, accompanied by an increase, decrease or no change in the level of spontaneous discharge. Cocaine interactions with

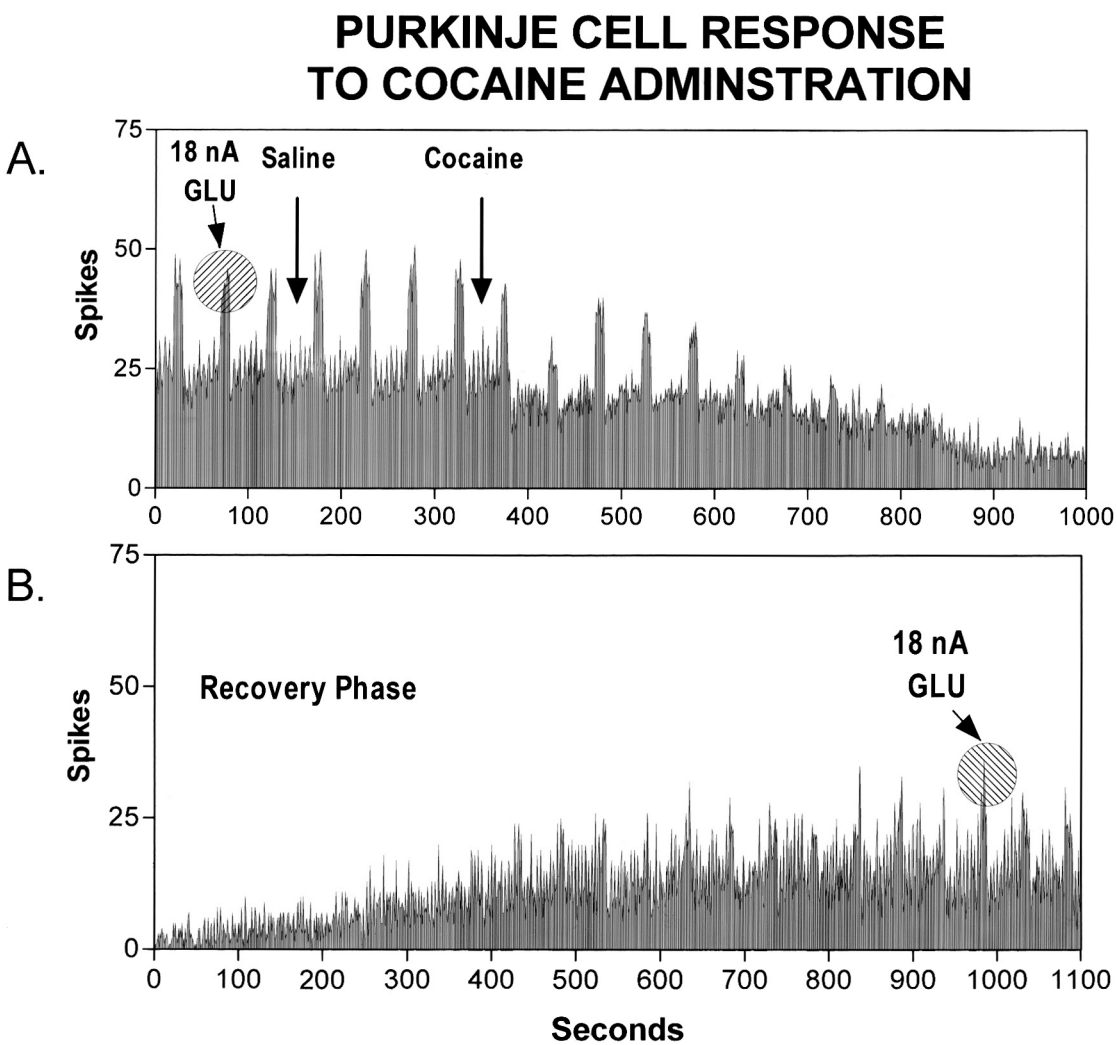

Fig. 1. (A) The figure shows a firing frequency histogram of a cerebellar Purkinje neuron. The effects of 1 mg/kg, i.v. cocaine administration are illustrated. Excitatory responses of an individual cell to microiontophoretic pulses (every 10 s) of glutamate (18 nA) were examined before and after a cocaine injection. A major inhibitory effect is observed during the first 2 min after cocaine application. Both spontaneous and evoked activities were decreased by cocaine. (B) There was a partial recovery of firing activity approximately 20 min after drug administration in which spontaneous activity recovers sooner and in greater magnitude than the glutamate-evoked excitation.

glutamate were termed "suppressive" when the evoked excitatory response was depressed proportionately more by drug application than was background discharge. Finally, cocaine was assumed to have no significant net effect on excitatory responses when spontaneous and evoked discharges were affected proportionately to the same extent during drug application. The latter case is represented by the dotted 45° "equivalence line" in the graph of Fig. 3. It should be noted that these definitions while providing a useful terminology make no assumptions concerning linear or non-linear summation effects.

3. Results

All animals in the present study were cocaine-naive. Only one cell and one drug dose were examined per animal. A total of 79 Purkinje neurons were tested in the study. Fig. 1A illustrates the effect of cocaine (1 mg/kg, i.v.) on glutamate-induced excitation and spontaneous activity of a single Purkinje neuron. In this particular case, the maximal effect was observed 10 min after drug injection. However, for the sample of cells tested the maximum response was observed from 2–10 min post-drug with complete or partial return to pre-drug levels of responsiveness 30 min after peak effect. For the case shown in Fig. 1, cocaine decreased both spontaneous and glutamate-evoked activity immediately following drug administration. The spontaneous firing rate of this cell was reduced 25.7%, from 26 to 19.33 spikes/s. At the same time, glutamate-evoked discharge was suppressed, from 47.7 to 38.3 spikes/s, a reduction of 19.58%. By contrast, following a control injection of saline spontaneous activity was reduced 10.3%, from 26 to 23.33 spikes/s, whereas glutamate-evoked excitation was increased 4.0%, from 47.7 to 49.6 spikes/s. Partial recovery of both spontaneous and evoked discharge was observed 20 min after drug administration with spontaneous activity recovering to control levels more quickly than glutamate-evoked responses.

As illustrated by the example in Fig. 2, procaine, an analogue of cocaine with similar local anesthetics properties, did not alter spontaneous or glutamate-evoked discharge of Purkinje neurons. For the cell shown, neither spontaneous activity (increased 5.3% from 20.66 to 21.83 spikes/s) nor glutamate-evoked firing (decreased 5.7% from 35.33 to 33.33 spikes/s) was affected significantly following procaine injection.

To differentiate between cocaine's actions on spontaneous activity and glutamate-induced discharge, an analysis using previously established criteria (see Methods) was carried-out. The results demonstrate that in a majority of cells tested cocaine had no differential effect on glutamate-induced excitation. The majority of cocaine cells can be observed in the lower left quadrant in the summary graph of changes in spontaneous vs. glutamate-evoked activity indicating an equal suppression of both evoked and spontaneous firing (Fig. 3). With higher doses (1 mg/kg), an increase in suppression was observed (25%); whereas with low doses (0.25 mg/kg), an elevation in absolute potentiation was revealed (Table 1). It is interesting to note that procaine at doses of 1 mg/kg, i.p. had mainly no differential effects on glutamate excitation al-

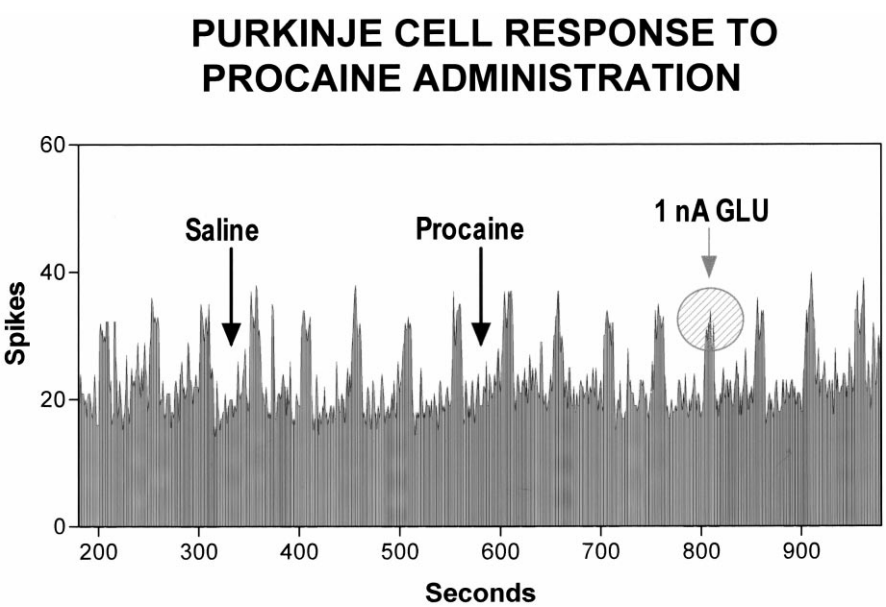

Fig. 2. The figure illustrates a Purkinje cell response to an i.v. injection of the local anesthetic procaine (1 mg/kg, i.v.). Glutamate pulses were given every 40 s at 1 nA. It can be observed that neither an isovolumetric saline injection nor procaine administration had any effect on spontaneous activity or glutamate-evoked excitation.

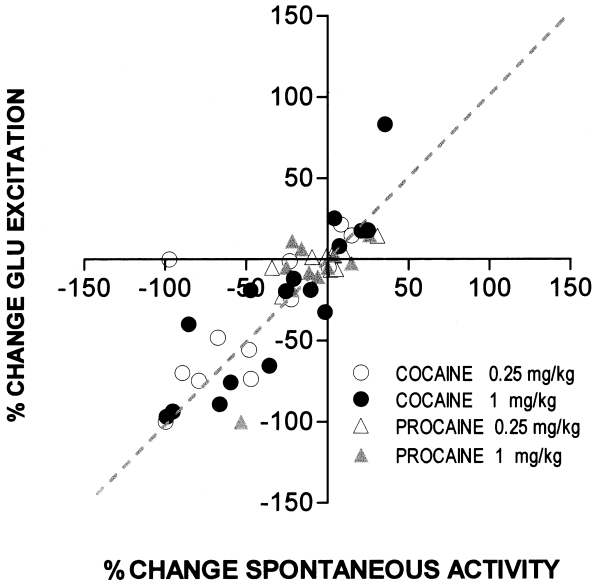

Fig. 3. Summary of cocaine effects on glutamate-evoked excitation and spontaneous activity in animals after cocaine or procaine administration. Points that lie below the 45° equivalence line indicate cases where cocaine suppressed glutamate-evoked responses more than spontaneous activity, thus yielding a net reduction in cell responsiveness to glutamate. Cocaine was assumed to have no significant net effects on excitatory responses when spontaneous and evoked discharges were affected proportionately to the same extent during drug administration. This is represented by the dotted 45° equivalence line in the graph. The majority of cocaine-treated cells can be observed in the lower left quadrant indicating an equal suppression of both evoked and spontaneous firing. Each symbol represents one cell.

though an absolute potentiation of some cells (27.3%) was also be observed. The latter finding is in agreement with studies already completed in rat cerebellum indicating that locally applied procaine (i.e. by iontophoresis) can produce a net enhancement of glutamate excitation, while at higher doses than cocaine (Jimenez-Rivera and Waterhouse, unpublished). These results are in accordance with recent investigation, which suggests that procaine could have psychostimulatory properties and animals can even self-administer the drug (Servan-Schreiber et al., 1998; Wilcox et al., 1999).

The activity of 28 Purkinje neurons was recorded before and after intravenous cocaine (1.0 mg/kg; $n = 16$; 0.25 mg/kg; $n = 12$) administration (Fig. 4A and B, top panel). Analysis of the data from this sample of cells showed that spontaneous firing rate was decreased significantly after cocaine administration at both concentrations. At 1.0 mg/kg, i.v. cocaine reduced spontaneous activity from 25.01 ± 2.11 to 18.89 ± 3.33 spikes/s ($\pm$S.E.M.). This change was statistically significant ($t = 2.35$, $P < 0.033$). At 0.25 mg/kg, i.v. cocaine also produced a significant ($t = 2.96$, $P < 0.01$) reduction in spontaneous activity from 23.4 ± 2.62 to 13.2 ± 3.49 spikes/s ($\pm$S.E.M.). Excitatory responses to glutamate were also significantly reduced after injection of cocaine at 1 mg/kg, i.v. (from 41.9 ± 3.35 to 30.1 ± 5.02 spikes/s ($\pm$S.E.M.); $t = 2.16$, $P < 0.047$) and 0.25 mg/kg, i.v. (from 37.9 ± 3.86 to 27.2 ± 6.47 ($\pm$S.E.M.); $t = 2.96$, $P < 0.01$).

The activity of 21 Purkinje neurons was recorded after intravenous procaine administration (1.0 mg/kg; $n = 11$; 0.25 mg/kg; $n = 10$). The bottom of Fig. 4A shows the population analysis of the changes in firing activity after 1 mg/kg, i.v., procaine administration. It can be observed that there were no changes in spontaneous firing after drug injection (from 23.35 ± 2.40 to 20.54 ± 2.27 spikes/s; $t = 1.57$, $P < 0.15$). Similarly, there were no changes in glutamate-evoked activity after procaine injections (from 34.74 ± 2.7 to 30.68 ± 4.16 spikes/s; $t = 1.03$, $P < 0.33$). The results show no change in either spontaneous activity after 0.25 mg/kg, i.v. procaine (from 23.5 ± 2.22 to 24.8 ± 3.72 spikes/s; $t = 0.24$, $P < 0.81$) nor in glutamate-evoked discharges (from 39.0 ± 3.54 to 39.9 ± 3.72 spikes/s; $t = 0.29$, $P < 0.77$).

In order to determine if cocaine effects were mediated by catecholamines, nine animals were injected with reserpine (5 mg/kg, i.p.) 5 h before recording sessions. The graph in Fig. 5 illustrates the effects of cocaine (1 mg/kg, i.v.) on Purkinje neuron spontaneous and glutamate-evoked discharge in animals pre-treated with reserpine. Following reserpine pre-treatment, cocaine did not alter Purkinje cell spontaneous activity (from 22.7 ± 1.67 to 19.5 ± 2.74 spikes/s; $t = 2.12$, $P < 0.07$), however, glutamate-evoked excitation was still reduced by cocaine (from 33.0 ± 2.25 to 29.4 ± 3.14 spikes/s; $t = 2.78$, $P < 0.02$) in these animals.

Since the results using reserpine suggested that catecholamines are at least partially involved in the effects induced by cocaine in the cerebellum, experiments were carried out to identify the adrenergic receptor moiety that mediates such actions. As was discussed previously, the

Table 1
Purkinje neuronal responsiveness to glutamate-induced excitation

Treatment	Absolute potentiation	Enhancement	Suppression	No differential change
Cocaine (1 mg/kg, i.v.) $n = 16$	2 (12.5%)	2 (12.5%)	4 (25%)	8 (50%)
Cocaine (0.25 mg/kg, i.v.) $n = 12$	4 (33.3%)	1 (8.3%)	1 (8.3%)	6 (50%)
Procaine (1 mg/kg, i.v.) $n = 11$	3 (27.3%)	0	1 (9%)	7 (63.3%)
Procaine (0.25 mg/kg, i.v.) $n = 10$	0	0	1 (10%)	9 (90%)

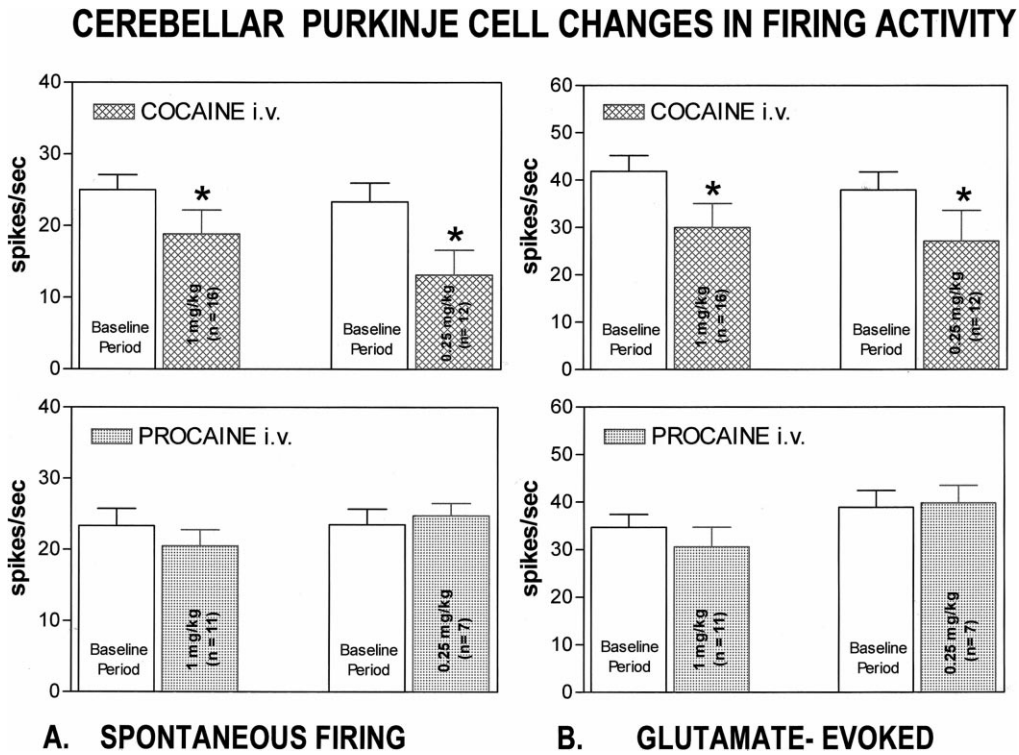

Fig. 4. This figure shows cocaine and procaine's actions on Purkinje cell activity. Bar graphs depict the means ± S.E.M of firing activity. Baseline periods include activity during the first 200 s. A significant reduction in spontaneous firing occurs only after cocaine administration and not after procaine injections (left side). The glutamate-evoked excitation was only suppressed by cocaine administration. Procaine had no effect on the glutamate-evoked excitation. Asterisks denote a statistically significant change in firing frequency ($P < 0.05$ paired-sample *t*-test).

cerebellum receives a prominent noradrenergic input from the locus coeruleus (Moore and Bloom, 1979; Hoffer et al., 1973). The release of high concentrations of norepinephrine in the cerebellum has been shown to produce a decrease in Purkinje cell activity (Hoffer et al., 1971; Woodward et al., 1991a,b; Moises et al., 1983). Therefore, we focused our attention on the potential role of noradrenergic receptors in mediating cocaine's suppressant effects.

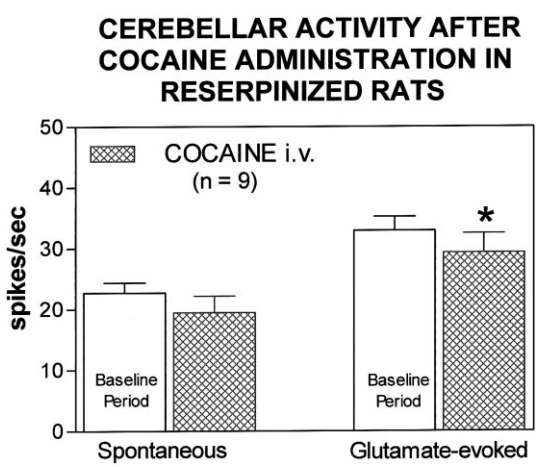

Fig. 5. The figure shows the effects of cocaine administration (1 mg/kg, i.v.) in animals that were treated with reserpine (5 mg/kg, i.p.) 5 h prior cocaine injections. Reserpine treatment was able to block cocaine's actions on Purkinje cell spontaneous activity. Cocaine still was capable of inhibiting glutamate-evoked excitation under these conditions. Asterisks denote a statistically significant change in firing frequency ($P < 0.05$ paired-sample *t*-test).

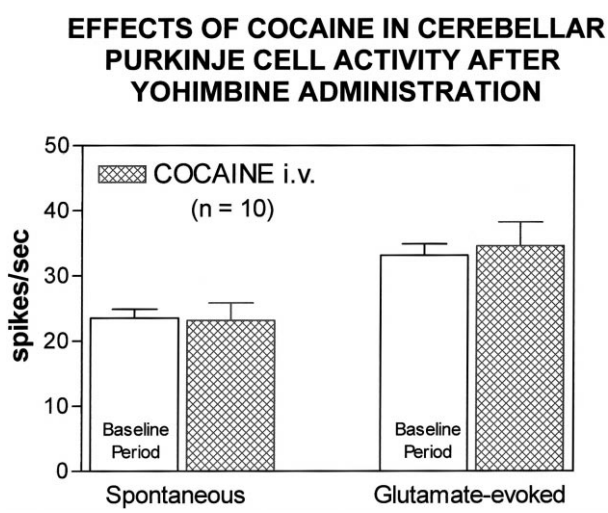

Fig. 6. The figure depicts the effects of yohimbine, a selective α_2-adrenoceptor antagonist, on cocaine actions in the cerebellum. Yohimbine (5 mg/kg, i.p.), was given intraperitoneally 10 min before cocaine administration (1 mg/kg, i.v.). The drug was able to suppress both, cocaine's action on spontaneous activity and on glutamate-evoked excitation.

An initial group ($n = 10$) of animals were treated with yohimbine (5 mg/kg, i.p.), an α_2-adrenoceptor blocker, 10–15 min before 1 mg/kg, i.v. cocaine administration (Fig. 6). Yohimbine pre-treatment completely blocked cocaine's inhibitory actions on Purkinje cells, i.e. no significant changes were observed in spontaneous activity (from 23.9 ± 1.46 to 23.2 ± 2.71 spikes/s; $t = 0.29$, $P < 0.78$) or glutamate-induced excitation (from 33.2 ± 1.74 to 34.6 ± 3.68 spikes/s; $t = 0.49$, $P < 0.63$) following cocaine administration in yohimbine pre-treated animals. Yohimbine by itself did not have any effect on spontaneous (from 23.90 ± 1.46 to 24.52 ± 1.85 spikes/s; $t = 0.48$, $P < 0.65$) or glutamate-evoked excitation (from 33.2 ± 1.74 to 36.05 ± 1.99 spikes/s; $t = 1.49$, $P < 0.17$) when compared to the non-drug baseline period. These results suggest an involvement of α_2-adrenoceptors in cocaine-induced inhibition of Purkinje cell discharge. α_1-Adrenoceptor blockers (i.e. prazosin) were not employed in the study since they have been mainly associated with enhancement of neuronal excitation (Mouradian et al., 1991) and not with inhibition of cell firing, which was cocaine's main effect.

In order to investigate the involvement of other noradrenergic receptors in cocaine-induced inhibition, another group of animals ($n = 8$) were treated with the β-adrenoceptor blocker propranolol (10 mg/kg, i.p., $n = 8$) 10 to 15 min before cocaine (1 mg/kg, i.v.) administration. Again, cocaine was able to significantly reduce both the spontaneous activity (from 18.7 ± 0.67 to 9.65 ± 2.98 spikes/s; $t = 2.89$, $P < 0.02$) and the glutamate-induced excitation (from 27.9 ± 0.91 to 15.8 ± 4.61 spikes/s; $t = 2.71$, $P < 0.03$) suggesting that β-adrenoceptors are not directly involved in cocaine's effects on Purkinje cell activity (Fig. 7).

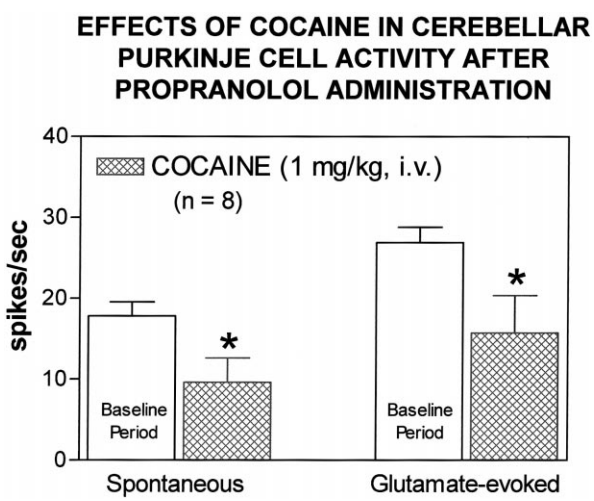

Fig. 7. The figure demonstrates the effects of cocaine administration on cerebellar Purkinje cell activity after propranolol (10 mg/kg, i.p.), injections. Propranolol was injected 10–15 min prior cocaine administration. The β-adrenoceptor antagonist was not able to block cocaine's inhibitory actions on either spontaneous or glutamate-evoked discharges. Asterisks denote a statistically significant change in firing frequency ($P < 0.05$ paired-sample t-test).

4. Conclusions

The major goal of the present investigation was to study the effects of intravenous cocaine administration on Purkinje cell excitation. The cerebellar Purkinje neuronal system provides an excellent model for the study of cocaine actions in the central nervous system (CNS) and norepinephrine physiology due to the well-established noradrenergic connections (Woodward et al., 1991a,b). Similar to previous investigations, our strategy was to explore potential non-dopaminergic actions of cocaine in central circuits using the cerebellar Purkinje neuron as an electrophysiological test system (Waterhouse et al., 1991). The present investigation demonstrated that intravenous cocaine administration decreases Purkinje cell spontaneous activity and glutamate-evoked discharge by a mechanism involving α_2-adrenoceptor activation.

4.1. Electrophysiological effects of cocaine

In the present study, the main action of cocaine was a reversible suppression of both spontaneous activity and glutamate-induced excitation of Purkinje neurons. Neither of these actions was consistently observed following volumetric equivalent injections of NaCl. The effects appear to be selective for cocaine since procaine, a closely related local anesthetic substance, was ineffective in producing similar actions. These results, together with the fact that no reduction in spike amplitude of the cells was observed, argue against a local anesthetic effect of cocaine.

As described in a previous report from our laboratory (Waterhouse et al., 1991), we were not able to confirm a consistent excitatory action of cocaine on rat cerebellar Purkinje neuron discharge (Pitts and Marwah, 1987); despite the fact that only anesthetic (chloral hydrate vs. urethane) and route of drug administration (i.v. vs. i.p.) were different between the two investigations.

4.2. Mechanisms of action

Since microiontophoretic application and synaptic release of norepinephrine have been shown previously (Moises et al., 1983; Woodward et al., 1991a,b) to induce suppression of Purkinje cell spontaneous activity, reserpine was employed to assess the possible role of catecholamines on cocaine's action in the cerebellum. Reserpine inhibits the storage of biogenic amines (dopamine, norepinephrine and 5-HT) in intracellular vesicles and thus causes depletion of these monoamines from nerve terminals (Katzung, 1995). The results of our study showed that reserpine pre-treatment blocked the effects of cocaine on spontaneous activity but not on glutamate-evoked excitation. Thus, while these findings provide evidence of catecholamine involvement in cocaine-induced inhibition, we

could not confirm catecholamine involvement in cocaine-mediated enhancement of glutamate excitation.

The effect of reserpine in the present study might not be surprising since the drug has non-selective actions on several catecholamines (see Katzung, 1995). It is possible that the relative synaptic concentrations of catecholamines (i.e. dopamine and norepinephrine) achieved after reserpine treatment could have antagonistic actions impinging on glutamate transmission as has been shown before (Zheng et al., 1999). It was demonstrated that dopamine, at low concentrations (< 50 μM), enhanced an NMDA-mediated current in cortical cells whereas at higher concentrations, there was a net suppressive effect.

The locus coeruleus is the major source of noradrenergic projections to the cerebellar cortex (Foote et al., 1983). Activation of this pathway has been demonstrated to induce inhibition of Purkinje cell activity (Hoffer et al., 1973). α_2-Adrenoceptor activation, in general, has been associated with suppression of neuronal discharge following synaptic release of norepinephrine (Katzung, 1995). To assess the potential role of α_2-adrenoceptor in mediating cocaine-induced suppression of Purkinje cell firing, animals were treated with yohimbine, an α_2-adrenoceptor antagonist, prior to cocaine injections. Yohimbine administration blocked cocaine-induced suppression of spontaneous activity and glutamate-evoked excitation.

In previews studies, α_2-adrenoceptors have been localized in the cerebellum of rats and humans (Alburges, et al., 1993; De Vos et al., 1994). Other experiments have demonstrated that activation of α_2-adrenoceptors inhibit Purkinje neuron spontaneous activity. This inhibitory effect was mimicked by the selective α_2-adrenoceptor agonist clonidine and blocked by idazoxan but not by timolol or prazosin (Parfitt et al., 1988). An investigation of the intracellular mechanisms involved in norepinephrine modulatory actions in the cerebellum concluded that norepinephrine's inhibitory actions in this structure are mediated by an α_2-adrenoceptor linked to a cAMP system coupled to an inhibitory G protein (Mori-Okamoto et al., 1991). Therefore, our results suggest that cocaine by inhibiting the norepinephrine's reuptake mechanism, increase this transmitter at the synaptic cleft where it binds to an α_2-adrenoceptor at the Purkinje cell surface triggering the above mentioned intracellular actions. The observed inhibitions of spontaneous and evoked activity after cocaine administration could be the result of the activation of the aforementioned intracellular mechanisms. Further studies should be directed to test this hypothesis.

Since β-adrenoceptors are known to be involved in noradrenergic enhancement of GABA inhibition in the cerebellum (Sessler et al., 1989), we administered the β-adrenoceptor antagonist propranolol before cocaine injections. However, propranolol was ineffective in reducing cocaine's inhibitory actions on Purkinje cell spontaneous and glutamate-evoked activity. This result provides evidence, although indirect, against a possible involvement of an enhanced GABAergic inhibition in the suppression of Purkinje cell discharge after cocaine administration. Based on our previous investigations (Waterhouse et al., 1991), we should expect a facilitation of the GABAergic system after cocaine injections. Nevertheless, the role of GABA inhibition after intravenous cocaine administration was not directly studied in the present investigation.

4.3. Possible behavioral and cellular correlates of cocaine

Cocaine's rewarding effects have been mainly attributed to its action on dopaminergically innervated circuits (Leshner and Koob, 1999). However, cocaine has other psychostimulant effects that are not readily accounted for by interactions with dopamine pathways. In particular, it has been shown that cocaine can exert potent modulatory actions on sensory information processing, yet primary sensory pathways are not directly innervated by dopamine systems. It has been suggested that these effects are mediated by cocaine influences on norepinephrine and 5-HT reuptake (Jimenez-Rivera and Waterhouse, 1991; Waterhouse et al., 1996; Bekavac and Waterhouse, 1995; Walsh and Cunningham, 1997). In experimental animals, it has also been shown that cocaine injections can increase locomotor activity and induce stereotyped behaviors (Robinson and Berridge, 1993). These actions of the drug are not as well characterized in terms of the neural circuits or neurotransmitters involved. The cerebellum is a brain area classically associated with control and coordination of movement (Llinás and Walton, 1990). However, recent investigations have shown that the cerebellum has other important functions related to cognition and learning of motor and non-motor tasks (Drepper et al., 1999; Thompson et al., 1998; Parsons et al., 1997). In terms of cognition, lesions of the vermis and posterior lobe of the cerebellum produce prominent behavioral changes in patients characterized by impairments of executive functions such as motor planning, set shifting and verbal fluency. In addition, there are changes in personality including blunting of affect or disinhibited behavior. This constellation of effects induced by cerebellar lesions has been called the "cerebellar cognitive affective syndrome" (Schmahmann and Sherman, 1998). Moreover, it has been suggested that this cerebellar syndrome involves an altered functional connection between the prefrontal, posterior parietal, temporal and limbic cortices and the cerebellum. It is well known that cocaine selectively influences the physiology of many of the aforementioned structures and also produces changes in behavior very similar to the ones mentioned above. As demonstrated in the present study, systemically administered cocaine completely altered the normal function of this structure, at least for the time the drug was acting in the CNS. Therefore, it is possible that acute administration of cocaine could produce a "functional lesion" of the cerebellum that leads to drug-related alterations in overt behavior and cognition.

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