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Hippocampal noradrenergic neurotransmission in concurrent EEG desynchronization and inhibition of penile erection induced by cocaine in the rat

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1 We previously reported that cocaine may induce activation of cortical (cEEG) and hippocampal (hEEG) electroencephalographic signals, concurrent with inhibition of penile erection, *via* an action on the hippocampal formation. The present study further evaluates the role of noradrenergic neurotransmission at the hippocampal formation in this process, using adult, male Sprague-Dawley rats anaesthetized and maintained by chloral hydrate.

2 Unilateral microinjection of cocaine (100 nmoles) into the hippocampal CA1 or CA3 subfield or dentate gyrus elicited significant activation of both cEEG and hEEG activity. At the same time, the intracavernous pressure (ICP), our experimental index for penile erection, underwent a discernible reduction.

3 Co-administration of equimolar doses (250 pmoles) of prazosin, naftopidil, yohimbine or rauwolscine significantly reversed those effects elicited by cocaine on cEEG, hEEG and ICP.

4 Microinjection unilaterally of equimolar doses (5 nmoles) of norepinephrine, phenylephrine or BHT 933 into the hippocampal formation, similar to cocaine, also induced appreciable cEEG and hEEG excitation, with a simultaneous decrease in ICP.

5 We conclude that cocaine may activate cEEG and hEEG and decrease ICP *via* noradrenergic neurotransmission, possibly engaging at least $\alpha_{1A/D}$-, α_{2B}- and α_{2C}-adrenoceptors at the hippocampal formation.

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Keywords: Cocaine; EEG activation; penile erection; hippocampal formation; noradrenergic neurotransmission; α_1- and α_2-adrenoceptors

Abbreviations: cEEG, cortical electroencephalogram; hEEG, hippocampal electroencephalogram; ICP, intracavernous pressure; MPF, mean power frequency; NE, norepinephrine; RMS, root mean square

Introduction

It is well-known from animal and human studies (Wallach & Gershon, 1971; Altshuler *et al*., 1976; Herning *et al*., 1985; Yabase *et al*., 1990) that, as a central nervous stimulant (Van Dyke & Byck, 1977), cocaine induces electroencephalographic (EEG) desynchronization. Acute use of cocaine may also increase sexual behaviour as an aphrodisiac (Cohen, 1975), and has been associated with penile erection (Cregler & Mark, 1986) or priapism (Fiorelli *et al*., 1990; Rodriguez-Blazquez *et al*., 1990). However, chronic use of cocaine may result in difficulties in maintaining an erection and ejaculation or impotence (Siegel, 1982; Smith *et al*., 1984; Cocores *et al*., 1986; Cregler & Mark, 1986).

Whether the effects of cocaine on EEG activity and penile erection are inter-related is essentially unknown. Recent work from our laboratory (Chang *et al*., 1998b) provides the first clue that cocaine may affect cortical EEG activity and penile erection by acting on a common neural substrate. Based on evaluations of concurrent changes in EEG activity and intracavernous pressure, an experimental index for penile erection (Chen *et al*., 1992), in conjunction with intravenous or hippocampal application of cocaine, we conclude that cocaine may induce cortical EEG desynchronization but cause a reduction in penile erection *via* an action on the hippocampal formation.

The neurotransmitter mechanisms that underlie the differential participation of the hippocampal formation in cocaine-induced excitation of cortical EEG and inhibition of penile erection remain unexplored. One possible candidate is noradrenergic neurotransmission. As an inhibitor of monoaminergic neurotransmission, cocaine increases extracellular norepinephrine (NE) (Hernandez *et al*., 1988) by blocking its presynaptic reuptake (Langer & Enero, 1974). More importantly, noradrenergic innervation of the hippocampal formation, principally from the locus coeruleus (Loy *et al*., 1980), has been reported. Binding studies (Biegon *et al*., 1982; Dooley & Bittiger, 1982) revealed that the majority of adrenoceptors present in this forebrain structure belongs to the α_1 and α_2 subtypes, with only a minor presence of β-adrenoceptors.

The present study was carried out against the above background to address two issues regarding the concurrent EEG desynchronization and inhibition of penile erection induced by cocaine. First, is noradrenergic neurotransmission at the hippocampal formation involved in the process? Second, what are the subtypes of α-adrenoceptors that may be engaged? We found that cocaine may indeed activate both

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cortical and hippocampal EEG and decrease ICP *via* noradrenergic neurotransmission, possibly engaging at least $\alpha_{1A/D}$-, α_{2B}- and α_{2C}-adrenoceptors at the hippocampal formation.

Methods

Animals and general procedures

The experimental procedures used in this study were approved by the Institutional Committee on Experimental Animals. All efforts were made to minimize animal suffering, and to reduce the number of animals used.

Adult, male Sprague-Dawley rats (220–270 g) obtained from the Experimental Animal Center, National Science Council, Taiwan, Republic of China, were used. They were housed in a temperature- ($24\pm1^{\circ}C$) and light- (12 : 12 h light:dark cycle, lights on from 0800 h) controlled animal room, with free access to food and water.

Experiments were carried out during the day in rats that were anaesthetized initially with chloral hydrate (400 mg kg^{-1}, i.p.). The left femoral artery and vein were routinely cannulated for the measurement of systemic arterial pressure and maintenance of anaesthetic level by intravenous infusion of chloral hydrate (40 mg kg^{-1} h^{-1}). Systemic arterial pressure was recorded through a pressure transducer (Gould P23XL, Valley View, OH, U.S.A.) and an universal amplifier (Gould 20-4615-58). Heart rate was derived from the systemic arterial pressure signals (Yang *et al*., 1996). The trachea was intubated to maintain patency of the airway. Following the completion of surgery, the animal was fixed to a stereotaxic headholder (Kopf 1404, Tujunga, CA, U.S.A.), and the rest of the body was placed on a heating pad to maintain body temperature at 37°C throughout the experiment.

Recording and quantification of EEG activity

As in our previous studies (Chang *et al*., 1994; 1995; 1996; 1998a,b), EEG signals were recorded bilaterally and differentially with two stainless steel screws implanted epidurally above the primary somatosensory cortex (1 mm posterior to the bregma and 5 mm lateral to the midline). EEG signals from the right side of the hippocampal formation were concurrently recorded *via* a stereotaxically positioned bipolar concentric electrode (Rhodes Medical SNE-100, Woodland Hills, CA, U.S.A.) at: 2.3–3.2 mm posterior to the bregma, 3.6–4.4 mm from the cortical surface, 1.5–2.4 mm lateral to the midline (Chang *et al*., 1998a,b). Bioelectric signals were amplified and filtered by an universal amplifier (Gould 20-4615-58), digitized (Adaptec AHA-1520A, Milpitas, CA, U.S.A.), and stored on magneto-optical disk (Kyocera FRE-3651W-5P, Kyoto, Japan). The response range (1–300 Hz) of the system amply covered the range of EEG frequencies (1–32 Hz) in which we were interested.

Both cortical and hippocampal (cEEG and hEEG) signals were subject to continuous, on-line and real-time spectral analysis (Chang *et al*., 1994; 1995; 1996; 1998a,b). In particular, we quantified the magnitude of EEG activity by calculating the root mean square (RMS) value. The frequency domain of EEG signals was evaluated by the mean power frequency (MPF) of each spectrum. During the experiment, raw EEG signals were continuously displayed, in a real-time and on-line manner on a monitor and a printer, alongside RMS and MPF values, systemic arterial pressure and heart rate signals.

Recording of intracavernous pressure

The increase in intracavernous pressure (ICP) was used as our experimental index for penile erection (Chen *et al*., 1992). As reported previously (Chen *et al*., 1992; Chan *et al*., 1996; Chang *et al*., 1998a,b), the lower part of the body was rotated slightly to provide adequate exposure of the genital area. The skin overlying the penis was incised, and the prepuce was degloved to fully expose both corpora cavernosa. A 26-gauge needle filled with saline and connected to a Gould 23ID pressure transducer was carefully inserted into the corpus cavernosum on one side. Intracavernous administration of saline (250 μl) was routinely given at the beginning of the experiment to ensure the lack of leakage. Furthermore, papaverine (U-Liang Pharmaceuticals, Taiwan, Republic of China; 400 μg) was administered intracavernously at the end of each experiment to ensure that the ICP needle was lodged properly in the corpus cavernosum throughout the experiment, and that the carvernous tissues still responded adequately to this vasoactive agent used clinically to treat impotence (Sidi & Chen, 1987).

Microinjection of test agents into the hippocampal formation

Direct microinjection of test agents to the hippocampal formation on the left side (Chang *et al*., 1998a,b) was carried out with a stereotaxically positioned microsyringe needle connected to a 0.5-μl Hamilton microsyringe (Reno, NV, U.S.A.), using the same coordinates as in hEEG recording. A total volume of 50 nl was delivered over 1–2 min to allow for full diffusion of the injected solution. The temporal effects of each treatment on cEEG, hEEG, ICP, systemic arterial pressure and heart rate were evaluated for 60 min. In all cases, microinjection of the vehicle served as the volume control.

Test agents

Test agents used in the present study were freshly prepared during the experiment. These included cocaine (Department of Health, Taiwan, Republic of China), norepinephrine (Sigma, St. Louis, MO, U.S.A.), phenylephrine (Sigma), BHT 933 (RBI, Natick, MA, U.S.A.), prazosin (Sigma), yohimbine (Sigma), naftopidil (RBI) and rauwolscine (RBI). With the exception of naftopidil, which used 20% methanol as the solvent, all test agents were dissolved in aCSF.

Histology

At the conclusion of each experiment, the brain of the rat was removed and fixed in 30% sucrose in 10% formaldehyde-saline solution for at least 48 h. Histologic verifications of the microinjection or recording site in the hippocampal formation were carried out on frozen 25-μm sections stained with Neutral red.

Statistical analysis

All values are expressed as mean$\pm$s.e.mean. Differences between treatment groups were statistically assessed using one-way analysis of variance, followed by the Dunnett or Scheffé multiple range test for *a posteriori* comparison of means. $P<0.05$ was considered statistically significant.

Results

Effects of hippocampal application of cocaine on cEEG, hEEG or ICP

As we observed recently (Chang *et al*., 1998a), unilateral microinjection of cocaine (100 nmoles), together with either vehicle, into the hippocampal formation elicited significant changes in both cEEG and hEEG signals (Figures 1 and 2). There was a decrease in cEEG-RMS and an increase in cEEG-MPF values (Figure 2) that took place within 1–2 min after administration of cocaine, and persisted for 45–60 min (Figure 1). Whereas the hEEG signals manifested elevated RMS values (Figure 2), at a latency of 1–2 min and persisted throughout the duration of the 60-min recording (Figure 1), the MPF values did not undergo concomitant changes (Figures 1 and 2).

Interestingly, the ICP manifested an appreciable reduction, with a latency and duration comparable to that of changes in hEEG signals (Figures 1 and 2). As exemplified by Figure 1 in an animal that exhibited a waxing and waning baseline ICP, unilateral co-microinjection of cocaine and either vehicle resulted in a decrease in both the maximal and minimal amplitudes of the ICP waves. These changes in ICP were, however, not accompanied by discernible alterations in both systemic arterial pressure and heart rate (Table 1).

Effects of hippocampal application of α-adrenoceptor antagonists on cocaine-induced actions on cEEG, hEEG or ICP

Both activation of cEEG or hEEG signals and inhibition of ICP elicited by cocaine were significantly blunted when the α_1-adrenoceptor antagonist, prazosin (250 pmoles), was co-microinjected with cocaine (100 nmoles) into the hippocampal formation (Figure 2). Similar observations were obtained on co-administration of the α_2-adrenoceptor antagonist, yohimbine (250 pmoles), with cocaine (100 nmoles) into the hippocampal formation (Figure 2). The only difference was with cEEG-RMS values, which not only showed a reversal, but a discernible elevation. In both experiments, the systemic arterial pressure and heart rate exhibited minimal alterations (Table 1).

We are aware that in addition to high α_1 affinity, prazosin also binds to α_{2B} and α_{2C} receptors (see Bylund *et al*., 1994; Ruffolo *et al*., 1994). Likewise, apart from α_2-adrenoceptors, yohimbine may have a wide spectrum of affinities for other receptors, including imidazoline receptors (Hamilton, 1995). To evaluate whether these additional pharmacologic properties of prazosin and yohimbine may have confounded our results, we included in our study naftopidil, the specific $\alpha_{1A/D}$-adrenoceptor antagonist (Takei *et al*., 1999), and rauwolscine, the specific α_{2B}- and α_{2C}-adrenoceptor antagonist (Bylund *et al*., 1994) with low affinity for imidazoline receptors (Farsang & Kapocsi, 1999).

As depicted in Figure 2, co-microinjection of naftopidil (250 pmoles) with cocaine (100 nmoles) into the hippocampal formation resulted in changes in cEEG-MPF, hEEG-RMS, hEEG-MPF or ICP that were reminiscent of those produced by co-administration of prazosin with cocaine. The exception was with RMS values of cEEG signals, which were significantly reduced. The results from hippocampal application of rauwolscine (250 pmoles) and cocaine (100 nmoles), on the other hand, were comparable to those from co-microinjection of yohimbine with cocaine. Again, these changes were not accompanied by appreciable alterations in systemic arterial pressure and heart rate (Table 1).

Unilateral co-microinjection into the hippocampal formation of aCSF, together aCSF, 20% methanol or each α-adrenoceptor antagonist used in the present study, resulted in no significant changes in baseline RMS and MPF values of cEEG or hEEG signals, ICP, systemic arterial pressure or heart rate (data not shown).

Effects of hippocampal application of α-adrenoceptor agonists on cEEG, hEEG or ICP

The implied engagement of noradrenergic neurotransmission and both α_1- and α_2-adrenoceptors at the hippocampal formation in cocaine-induced activation of cEEG and hEEG and inhibition of ICP was further ascertained in our second series of experiments. Unilateral microinjection of NE (5 nmoles) to the hippocampal formation (Figure 3) elicited a significant decrease in cEEG-RMS and an appreciable increase in cEEG-MPF values. Both the RMS and MPF values of hEEG signals underwent a discernible elevation, along with an appreciable decrease in ICP. The systemic arterial pressure and heart rate, on the other hand, remained unaltered (Table 2).

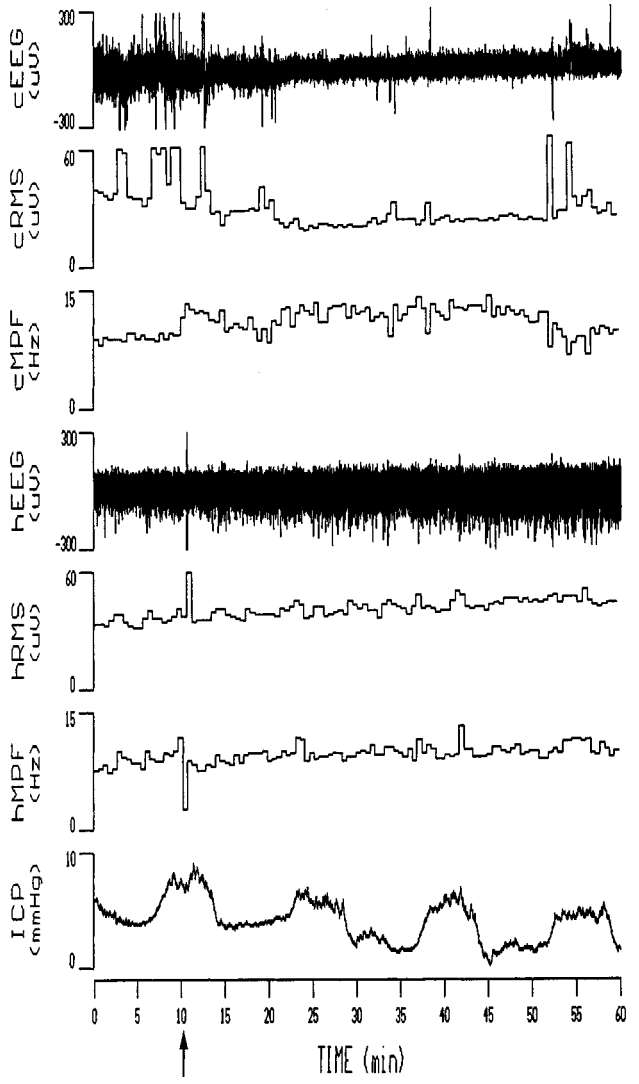

Figure 1 Illustrative example of the temporal effects of microinjection unilaterally into the hippocampal formation of cocaine (100 nmoles, at arrow), together with aCSF, on electroencephalographic signals recorded from the somatosensory cortex (cEEG) or hippocampal formation (hEEG), and their respective root mean square (cRMS or hRMS) and mean power frequency (cMPF or hMPF) values. Also shown are concurrent responses to cocaine of intracavernous pressure (ICP).

The specific α_1- and α_2-adrenoceptor agonists (Ruffolo *et al.*, 1991; Willems *et al.*, 1999), phenylephrine and BHT 933, were included to further decipher the participation of those two receptor subtypes in these processes. Microinjection unilaterally of BHT 933 (5 nmoles) into the hippocampal formation essentially duplicated the changes in cEEG, hEEG or ICP induced by NE (Figure 3). Whereas hippocampal application of phenylephrine (5 nmoles >) similarly produced an increase in cEEG-MPF or hEEG-RMS values and a decrease in ICP, there was an

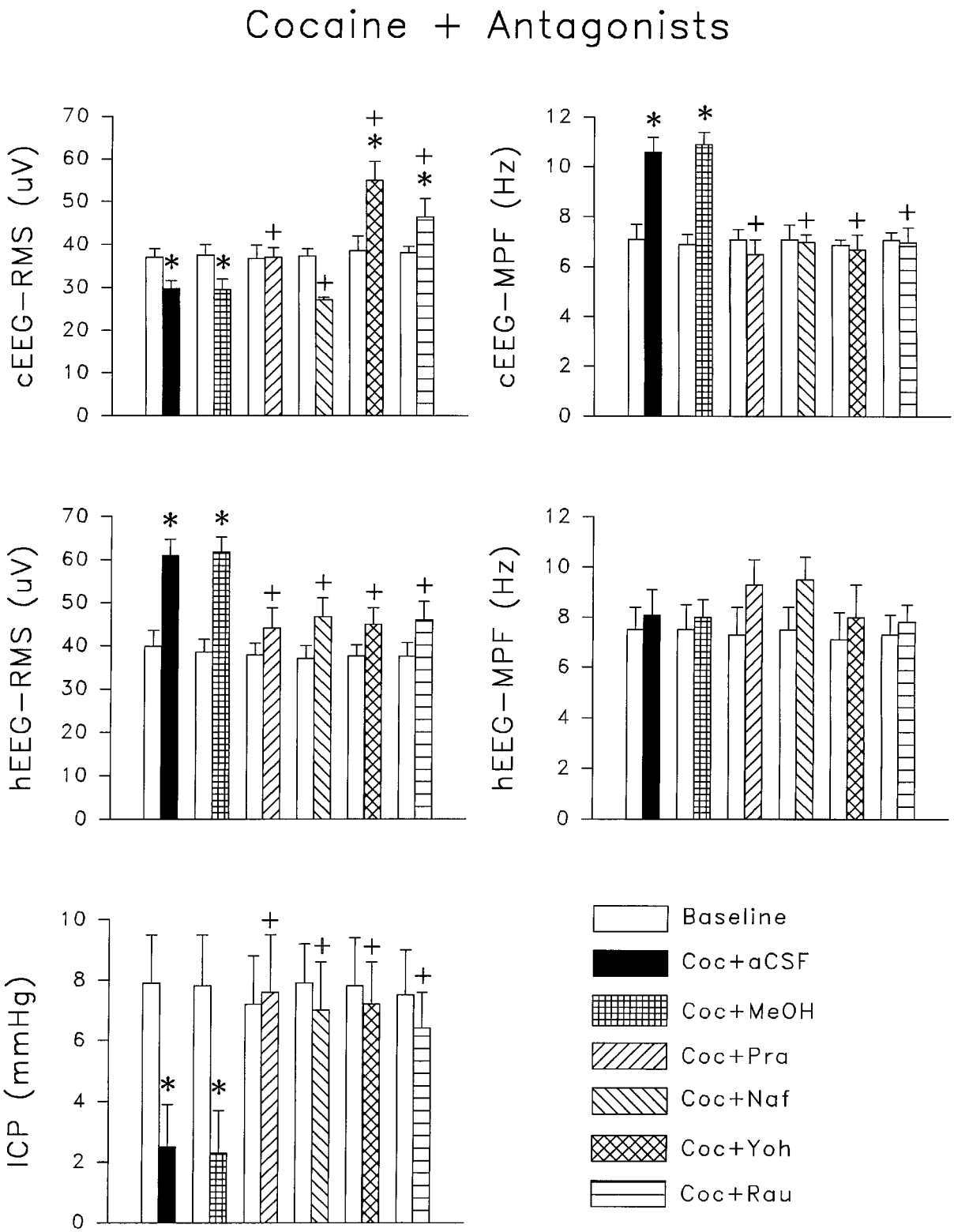

Figure 2 Maximal effects on root mean square (RMS) or mean power frequency (MPF) values of cortical or hippocampal electroencephalographic (cEEG or hEEG) signals and intracavernous pressure (ICP) of microinjection unilaterally into the hippocampal formation of cocaine (Coc, 100 nmoles), together with equimolar concentrations (250 pmoles) of prazosin (Pra), naftopidil (Naf), yohimbine (Yoh), rauwolscine (Rau), aCSF or 20% methanol (MeOH). The maximal values measured during 10 min of baseline recording and within 30 min after administration of Coc-Pra, Coc-Naf, Coc-Yoh, Coc-Rau, Coc-aCSF or Coc-MeOH are presented as mean $\pm$ s.e.mean, $n = 5-7$ animals per group. $^*P < 0.05$ vs baseline values in the Dunnett analysis. $^+P < 0.05$ vs the corresponding Coc-aCSF or Coc-MeOH group in the Scheffé analysis.

augmentation in cEEG-RMS and minimal changes in hEEG-MPF values (Figure 3). Again, neither treatment resulted in discernible alterations in systemic arterial pressure or heart rate (Table 2).

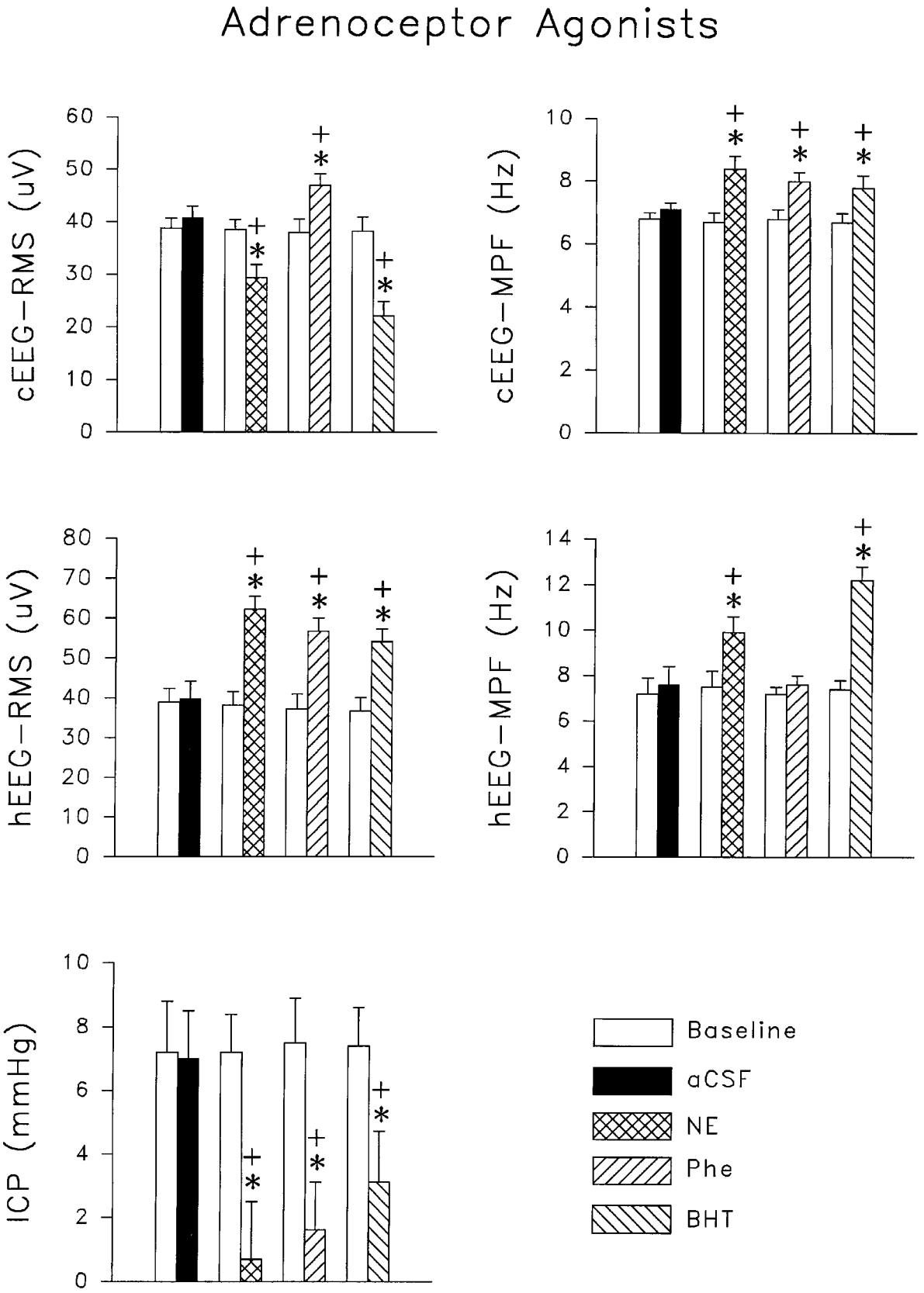

Figure 3 Maximal effects on root mean square (RMS) or mean power frequency (MPF) values of cortical or hippocampal electroencephalographic (cEEG or hEEG) signals and intracavernous pressure (ICP) of microinjection unilaterally into the hippocampal formation of equimolar concentrations (5 nmoles) of norepinephrine (NE), phenylephrine (Phe), BHT 933 (BHT) or aCSF. The maximal values measured during 10 min of baseline recording and within 30 min after administration of the agonists or aCSF are presented as mean ± s.e.mean, $n = 5-7$ animals per group. $^{*}P < 0.05$ vs baseline values in the Dunnett analysis, and $^{+}P < 0.05$ vs aCSF group in the Scheffé analysis.

Microinjection and recording sites in hippocampal formation

Histologic verifications (Figure 4) confirmed that our microinjection and recording sites were distributed randomly within CA1 or CA3 subfield or dentate gyrus of the hippocampal formation.

Table 1 Effects of cocaine administered with or without adrenoceptor antagonists on mean systemic arterial pressure or heart rate

	MSAP (mmHg)		*HR* (b.p.m.)	
	Before	*After*	*Before*	*After*
Coc + aCSF	77.6 ± 4.2	79.5 ± 3.8	368.2 ± 13.4	359.6 ± 10.5
Coc + MeOH	78.6 ± 4.7	75.7 ± 3.9	349.8 ± 14.2	356.2 ± 11.1
Coc + Pra	78.3 ± 3.0	74.2 ± 2.8	353.6 ± 13.4	348.8 ± 9.3
Coc + Naf	76.9 ± 3.4	78.2 ± 4.8	345.7 ± 13.2	353.5 ± 9.7
Coc + Yoh	77.8 ± 4.1	79.9 ± 3.2	354.7 ± 11.6	346.8 ± 11.2
Coc + Rau	78.1 ± 3.6	75.8 ± 3.7	349.2 ± 13.5	356.1 ± 12.4

Maximal effects on mean systemic arterial pressure (MSAP) or heart rate (HR) of microinjection unilaterally into the hippocampal formation of cocaine (Coc, 100 nmoles), together with equimolar concentrations (250 pmoles) of prazosin (Pra), naftopidil (Naf), yohimbine (Yoh), rauwolscine (Rau), aCSF or 20% methanol (MeOH). Values measured during 10 min of baseline recording and within 30 min after treatment are presented as mean ± s.e.mean, $n = 5-7$ animals per group. No significant difference ($P > 0.05$) exists among the treatment groups in one-way analysis of variance.

Table 2 Effects of adrenoceptor agonists on mean systemic arterial pressure or heart rate

	MSAP (mmHg)		*HR* (b.p.m.)	
	Before	*After*	*Before*	*After*
aCSF	76.5 ± 3.4	78.2 ± 4.3	347.3 ± 11.2	360.6 ± 11.9
NE	77.3 ± 3.7	79.1 ± 2.9	352.6 ± 10.8	371.5 ± 12.4
Phe	77.8 ± 4.1	75.6 ± 3.7	368.9 ± 11.6	378.4 ± 11.5
BHT	79.0 ± 3.6	77.6 ± 4.5	349.2 ± 12.1	362.5 ± 13.1

Maximal effects on mean systemic arterial pressure (MSAP) or heart rate (HR) of microinjection unilaterally into the hippocampal formation of equimolar concentrations (5 nmoles) of norepinephrine (NE), phenylephrine (Phe), BHT 933 (BHT) or aCSF. Values measured during 10 min of baseline recording and within 30 min after treatment are presented as mean ± s.e.mean, $n = 5-7$ animals per group. No significant difference ($P > 0.05$) exists among the treatment groups in one-way analysis of variance.

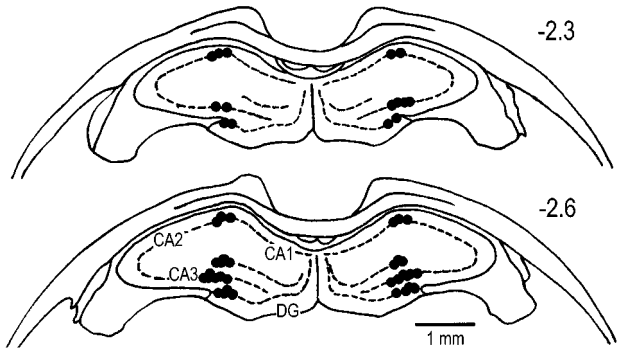

Figure 4 Diagrammatic representation of the hippocampal formation at two rostral-caudal levels showing the location of sites in the CA1 or CA3 subfield or dentate gyrus (DG) where microinjection of test agents or vehicle was delivered (left side); or hEEG signals were recorded (right side). For clarity, only 25% of the total microinjection or recording sites are included. Numbers on the right side of each diagram represent the distance from the bregma.

Discussion

We reported recently (Chang *et al*., 1998b) that the hippocampal formation may serve as a common neural substrate on which cocaine acts to induce concurrent activation of cEEG and hEEG signals and inhibition of penile erection. The present study extends these findings by demonstrating the involvement of noradrenergic neurotransmission at the hippocampal formation in this process, possibly engaging at least $\alpha_{1A/D}$-, α_{2B}- and α_{2C}-adrenoceptors.

Our observations that microinjection of either cocaine or NE into the hippocampal formation elicited similar actions on cEEG-RMS (decrease), cEEG-MPF (increase) or hEEG-RMS (increase) values strongly imply the crucial involvement of hippocampal noradrenergic neurotransmission in these events. The results from pharmacologic treatments further expound on this notion in light of our current understanding of the heterogeneity of α_1- and α_2-adrenoceptors (Bylund *et al*., 1994; Ruffolo *et al*., 1994). It is likely that α_1- and α_2-adrenoceptors at the hippocampal formation are respectively responsible for the increase and decrease in RMS values of cEEG signals. Whereas specific activation of α_1-adrenoceptors by microinjection of phenylephrine into the hippocampal formation elicited an increase, hippocampal application of the specific α_2-adrenoceptor agonist, BHT 933, induced a decrease in cEEG-RMS values. Similarly, cocaine promoted a reduction in the RMS values of cEEG signals on specific blockade of $\alpha_{1A/D}$-adrenoceptors at the hippocampal formation with naftopidil. On the other hand, an augmentation of cEEG-RMS values resulted on hippocampal administration of cocaine and the specific α_{2B}- and α_{2C}-adrenoceptor antagonist, rauwolscine. The differential participation of α_1- and α_2-adrenoceptors at the hippocampal formation in the regulation of the amplitude of cEEG signals is further supported by the lack of significant changes in cEEG-RMS values on co-microinjection of cocaine and the α_1-, α_{2B}- and α_{2C}-adrenoceptor antagonist, prazosin, into the hippocampal formation. The prevalence of α_2-adrenoceptor actions in this process is suggested when hippocampal administration of an equimolar dose of the non-specific α-adrenoceptor agonist, NE, also resulted in a reduction of the RMS values of cEEG signals, albeit less than that promoted by BHT 933.

Our results also indicate that $\alpha_{1A/D}$-, α_{2B}- and α_{2C}-adrenoceptors at the hippocampal formation are engaged in the enhancement of MPF values of cEEG signals. Hippocampal application of cocaine, NE, phenylephrine or BHT 933 induced comparable increases in cEEG-MPF values. Furthermore, the enhancing action of cocaine was uniformly blunted by prazosin, naftopidil, yohimbine or rauwolscine. Parallel conclusion can be drawn on the participation of these same adrenoceptor subtypes at the hippocampal formation in the augmentation of RMS values of hEEG signals. On the other hand, whereas microinjection of cocaine or phenylephrine induced minimal alterations in hEEG-MPF values, NE or BHT 933 produced a significant elevation. As such, a disparity exists in the actions of cocaine and α_1- or α_2-adrenoceptors in the hippocampal formation on the MPF values of hEEG signals. The pharmacologic significance of this dissimilarity, however, requires further elucidation.

Parallel changes in EEG activity from the hippocampal formation and cerebral cortex have been reported under physiologic or pharmacologic conditions. For example,

enhanced activity in the locus coeruleus increases EEG measures of arousal in the frontal cortex and hippocampus (Berridge & Foote, 1991). On the other hand, inhibition of locus coeruleus activity induces in the neocortex a shift from low-amplitude, high-frequency to large-amplitude, slow wave activity (Berridge *et al*., 1993). As the largest aggregate of NE-containing neurons in the brain (Grazanna *et al*., 1977), the locus coeruleus provides almost exclusively the noradrenergic innervations to the hippocampal formation (Ungerstedt, 1971; Segal & Landis 1974; Loy & Moore, 1979; Wyss *et al*., 1979, Loy *et al*., 1980, Foote *et al*., 1983). In addition, noradrenergic terminals are distributed uniformly within the entire hippocampal formation (Ishikawa *et al*., 1982). Binding sites identified in the hippocampal formation are primarily α_1- or α_2-adrenoceptors (Biegon *et al*., 1982; Dooley & Bittinger, 1982). It thus seems probable that cocaine may activate hEEG and cEEG signals by effecting an overflow of NE at the terminals of noradrenergic innervation from the locus coeruleus to the hippocampal formation, engaging at least $\alpha_{1A/D}$-, α_{2B}- and α_{2C}-adrenoceptors. Since β-adrenoceptors manifested only a minor presence in the hippocampal formation (Dooley & Bittinger, 1982), it is beyond the scope of the present study to explore their role in cocaine-induced cEEG and hEEG activation.

The enhanced noradrenergic neurotransmission by cocaine in the hippocampal formation may also underlie the elicited inhibition of penile erection. We recently demonstrated (Chang *et al*., 1998b) the existence of a novel mode of supraspinal modulations of penile erection in the form of negative feedback control via the hippocampal formation. We further noted that an increase in RMS values of the hEEG signals invariably accompanies an elevation in ICP, and may represent a trigger for this stipulated feedback inhibition on penile erection. Our present results indicate that microinjection of cocaine, NE, phenylephrine or BHT 933 into the hippocampal formation elicited an increase in hEEG-RMS values, along with a decrease in ICP. In addition, these effects of cocaine were significantly blunted by co-administration of prazosin, naftopidil, yohimbine or rauwolscine. These observations imply that cocaine may have triggered the descending limb of this subcortical feedback inhibitory mechanism on penile erection *via* noradrenergic neurotransmission at the hippocampal formation. In addition, the α-adrenoceptors involved may at least include the $\alpha_{1A/D}$, α_{2B} and α_{2C} subtypes.

Three observations confirmed the specificity of our experimental treatments. First, the lack of significant effects by aCSF or 20% methanol confirmed that the chemical properties of both solvents and the physical action of microinjection were not a confounding factor. Second, at the dose we used, microinjection of prazosin, naftopidil, yohimbine or rauwolscine into the hippocampal formation did not elicit discernible effects on baseline cEEG, hEEG or ICP. As such, the blunting actions of these α-adrenoceptor antagonists on the actions of cocaine may not be due simply to mutual cancellation of pharmacologic effects. Third, all our results were obtained under minimal alterations in systemic arterial pressure and heart rate. Thus, the observed changes in ICP, cEEG and hEEG signals may not be secondary to haemodynamic actions.

The provision of euphoria, heightened alertness, self-confidence and creativity, decrease in fatigue, and increase in sexual desire is believed to underlie the current trend of cocaine abuse (Brown, 1989; Commissaris, 1989; Johanson & Fischman, 1989). On the other hand, chronic users of cocaine reportedly experience difficulties in maintaining an erection and ejaculation or exhibit impotence (Siegel, 1982; Smith *et al*., 1984; Cocores *et al*., 1986; Cregler & Mark, 1986). Our present study demonstrates that noradrenergic neurotransmission at the hippocampal formation may at least provide the crucial premise for the concurrent activation of cEEG or hEEG signals and inhibition of penile erection induced by cocaine. Our results further indicate that at least the $\alpha_{1A/D}$-, α_{2B}- and α_{2C}-adrenoceptors in the hippocampal formation are engaged in these actions.

Local application of cocaine into the hippocampal formation increases extracellular concentration of NE (Thomas *et al*., 1994) or metabolism of this catecholamine (Robinson & Hambrecht, 1988). Cocaine also potentiates the responses of hippocampal neurons to NE (Yasuda *et al*., 1984). Approximately 80% of α_2-adrenoceptors are present as postjunctional signal transducing targets in the hippocampal formation (Heal *et al*., 1993). Furthermore, the postsynaptic α_2-adrenoceptors in the cortex and Edinger-Westphal nucleus of the rat are predominantly of the α_{2D} subtype, although this conclusion may not be extended to other brain areas (Heal *et al*., 1995). Thus, based on our current knowledge on the pharmacologic properties of the test agents we used, we conclude that the enhanced noradrenergic neurotransmission in the hippocampal formation induced by cocaine may engage at least postsynaptic $\alpha_{1A/D}$-, α_{2B}- and α_{2C}-adrenoceptors as the primary site of action for concurrent activation of hEEG and cEEG signals and inhibition of ICP.

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References

ALTSHULER, H.L., BURCH, N.R. & DOSSETT, R.G. (1976). The electroencephalographic effects of long term cocaine administration to rhesus monkeys. *Proc. Western Pharmacol. Soc.,* **19,** 323–326.

BERRIDGE, C.W. & FOOTE, S.L. (1991). Effects of locus coeruleus activation on electroencephalographic activity in neocortex and hippocampus. *J. Neurosci.,* **11,** 3135–3145.

BERRIDGE, C.W., PAGE, M.E., VALENTINO, R.J. & FOOTE, S.L. (1993). Effects of locus coeruleus inactivation on electroencephalographic activity in neocortex and hippocampus. *Neuroscience,* **55,** 381–393.

BIEGON, A., RAINBOW, T.C., MANN, J.J. & MCEWEN, B.S. (1982). Neurotransmitter receptor sites in human hippocampus: a quantitative autoradiographic study. *Brain Res.,* **247,** 379–382.

BROWN, R.M. (1989). Pharmacology of cocaine abuse. In *Cocaine, Marijuna, Designer Drugs: Chemistry, Pharmacology and Behavior*. eds. Redda, K.K., Walker, C.A. & Barnett, G. pp. 39–51, Boca Raton: CRC Press.

BYLUND, D.B., EIKENBERG, D.C., HIEBLE, J.P., LANGER, S.Z., LEFKOWITZ, R.J., MINNEMAN, K.P., MOLINOFF, P.B., RUFFOLO JR. R.R. & TRENDELENBURG, U. (1994). IV. International Union of Pharmacology nomenclature of adrenoceptors. *Pharmacol. Rev.,* **46,** 121–136.

CHAN, J.Y.H., HUANG, C.-L. & CHAN, S.H.H. (1996). Nitric oxide as a mediator of cocaine-induced penile erection in the rat. *Br. J. Pharmacol.,* **118,** 155–161.

CHANG, A.Y.W., CHAN, J.Y.H. & CHAN, S.H.H. (1998a). Participation of hippocampal formation in negative feedback inhibition of penile erection in the rat. *Brain Res.,* **788,** 160 – 168.

CHANG, A.Y.W., CHAN, J.Y.H. & CHAN, S.H.H. (1998b). Differential participation of hippocampal formation in cocaine-induced cortical electroencephalographic desynchronization and penile erection in the rat. *Synapse,* **30,** 140 – 149.

CHANG, A.Y.W., KUO, T.B.J. & CHAN, S.H.H. (1994). Power spectral analysis of electroencephalographic desynchronization induced by cocaine in the rat. *Neurosci. Lett.,* **170,** 175 – 178.

CHANG, A.Y.W., KUO, T.B.J., CHAN, J.Y.H. & CHAN, S.H.H. (1996). Concurrent elicitation of electroencephalographic desynchronization and penile erection by cocaine in the rat. *Synapse,* **24,** 233 – 239.

CHANG, A.Y.W., KUO, T.B.J., TSAI, T.-H., CHEN, C.-F. & CHAN, S.H.H. (1995). Power spectral analysis of electroencephalographic desynchronization induced by cocaine in the rat: correlation with evaluation of noradrenergic neurotransmission at the medial prefrontal cortex. *Synapse,* **21,** 149 – 157.

CHEN, K.K., CHAN, J.Y.H, CHANG, L.S., CHEN, M.T. & CHAN, S.H.H. (1992). Intracavernous pressure as an experimental index in a rat model for the evaluation of penile erection. *J. Urol.,* **147,** 1124 – 1128.

COCORES, J.A., DACKIS, C.A. & GOLD, M.S. (1986). Sexual dysfunction secondary to cocaine abuse in two patients. *J. Clin. Psychiatry,* **47,** 384 – 385.

COHEN, S. (1975). Cocaine. *J. Am. Med. Assoc.,* **231,** 74 – 75.

COMMISSARIS, R.L. (1989). Cocaine: pharmacology and toxicology. In *Cocaine, Marijuana, Designer Drugs: Chemistry, Pharmacology and Behavior*. ed. Redda, K.K., Walker, C.A. & Barnett, G. pp. 71 – 81, Boca Raton: CRC Press.

CREGLER, L.L. & MARK, H. (1986). Medical complications of cocaine abuse. *New Engl. J. Med.,* **315,** 1495 – 1500.

DOOLEY, D.J. & BITTIGER, H. (1982). Characterization of neurotransmitter receptors in the rat hippocampal formation. *J. Neurochem.,* **38,** 1621 – 1626.

FARSANG, C. & KAPOCSI, J. (1999). Imidazoline receptors: from discovery to antihypertensive therapy (facts and doubts). *Brain Res. Bull.,* **49,** 317 – 331.

FIORELLI, R.L., MANFREY, S.J., BELKOFF, L.H. & FINKELSTEIN, L.H. (1990). Priapism associated with intranasal cocaine abuse. *J. Urol.,* **143,** 584 – 585.

FOOTE, S.L., BLOOM, F.E. & ASTON-JONES, G. (1983). Nucleus locus coeruleus: new evidence of anatomical and physiological specificity. *Physiol. Rev.,* **63,** 844 – 914.

GRAZANNA, R., MORRISON, J.H., COYLE, J.T. & MOLLIVER, M.E. (1977). Immunohistochemical demonstration of noradrenergic neurons in the rat brain: the use of homologous antiserum to dopamine-β-hydroxylase. *Neurosci. Lett.,* **4,** 127 – 134.

HAMILTON, C.A. (1995). Imidazoline receptors, subclassification, and drug-induced regulation. *Ann. N.Y. Acad. Sci.,* **763,** 57 – 65.

HEAL, D.J., BUTLER, S.A., PROW, M.R. & BUCKETT, W.R. (1993). Quantification of presynaptic α_2-adrenoceptors in rat brain after short-term DSP-4 lesioning. *Eur. J. Pharmacol.,* **249,** 37 – 41.

HEAL, D.J., CHEETHAM, S.C., BUTLER, S.A., GOSDEN, J., PROW, M.R. & BUCKETT, W.R. (1995). Receptor binding and functional evidence suggest that postsynaptic α_2-adrenoceptors in rat brain are of the α_{2D} subtype. *Eur. J. Pharmacol.,* **277,** 215 – 221.

HERNANDEZ, L., LEE, F. & HOEBEL, B.G. (1988). Microdialysis in the nucleus accumbens during feeding or drugs of abuse: amphetamine, cocaine, and phencyclidine. *Ann. N.Y. Acad. Sci.,* **537,** 508 – 511.

HERNING, R.I., JONES, R.T., HOOKER, W.D., MENDELSON, J. & BLACKWELL, L. (1985). Cocaine increase EEG beta: a replication and extension of Hans Berger's historic experiments. *EEG Clin. Neurophysiol.,* **60,** 470 – 477.

ISHIKAWA, K., OTT, T. & MCGAUGH, J.L. (1982). Evidence of dopamine as a transmitter in dorsal hippocampus. *Brain Res.,* **232,** 222 – 226.

JOHANSON, C.E. & FISCHMAN, M.W. (1989). The pharmacology of cocaine related to its abuse. *Pharmacol. Rev.,* **41,** 3 – 52.

LANGER, S.Z. & ENERO, M.A. (1974). The potentiation of responses to adrenergic nerve stimulation in the presence of cocaine: its relationship to the metabolic rate of released norepinephrine. *J. Pharmacol. Exp. Ther.,* **191,** 431 – 443.

LOY, R., KOZIELL, D.A., LINDSEY, J.D. & MOORE, R.Y. (1980). Noradrenergic innervation of the adult rat hippocampal formation. *J. Comp. Neurol.,* **189,** 699 – 710.

LOY, R. & MOORE, R.Y. (1979). Ontogeny of the noradrenergic innervation of the rat hippocampal formation. *Anat. Embryol.,* **157,** 243 – 253.

ROBINSON, S.E. & HAMBRECHT, K.L. (1988). The effect of cocaine on hippocampal cholinergic and noradrenergic metabolism. *Brain Res.,* **457,** 383 – 385.

RODRIGUEZ-BLAZQUEZ, H.M., CARDONA, P.E. & RIVERA-HERRERA, J.L. (1990). Priapism associated with the use of topical cocaine. *J. Urol.,* **143,** 358.

RUFFOLO JR. R.R., NICHOLS, A.J., STADEL, J.M. & HIEBLE, J.P. (1991). Structure and function of α-adrenoceptors. *Pharmacol. Rev.,* **43,** 475 – 505.

RUFFOLO JR. R.R., STADEL, J.M. & HIEBLE, J.P. (1994). α-Adrenoceptors: recent developments. *Med. Res. Rev.,* **14,** 229 – 270.

SEGAL, M. & LANDIS, S. (1974). Afferents to the hippocampus of the rat studied with the method of retrograde transport of horseradish peroxidase. *Brain Res.,* **78,** 1 – 15.

SIDI, A.A. & CHEN, K.K. (1987). Clinical experience with vasoactive intracavernous pharmacotherapy for treatment of impotence. *World J. Urol.,* **5,** 156 – 159.

SIEGEL, P.K. (1982). Cocaine and sexual dysfunction: the curse of mama coca. *J. Psychoactive Drugs,* **14,** 71 – 74.

SMITH, D.E., WESSON, D.R. & APTER-MARSH, M. (1984). Cocaine and alcohol induced sexual dysfunction in patients with addictive diseases. *J. Psychoactive Drugs,* **16,** 359 – 361.

TAKEI, R., IKEGAKI, I., SHIBATA, K., TSUJIMOTO, G. & ASANO, T. (1999). Naftopidil, a novel alpha 1-adrenoceptor antagonist, displays selective inhibition of canine prostatic pressure and high affinity binding to cloned human alpha 1-adrenoceptors. *Jap. J. Pharmacol.,* **79,** 447 – 454.

THOMAS, D.N., POST, R.M. & PERT, A. (1994). Focal and systemic cocaine differentially affect extracellular norepinephrine in the locus coeruleus, frontal cortex and hippocampus of the anaesthetised rat. *Brain Res.,* **645,** 135 – 142.

UNGERSTEDT, U. (1971). Stereotaxic mapping of the monoamine pathways in the rat brain. *Acta Physiol. Scand.,* **367** (Suppl.): 1 – 48.

VAN DYKE, C. & BYCK, R. (1977). Cocaine: 1884 – 1974. In *Cocaine and Other Stimulants*. eds. Ellinwood Jr. E.H. & Kilbey, M.M. pp. 1 – 30, New York: Plenum Press.

WALLACH, M.B. & GERSHON, S. (1971). A neuropsychopharmacological comparison of d-amphetamine, L-DOPA and cocaine. *Neuropharmacology,* **10,** 743 – 752.

WILLEMS, E.W., TRION, M., DE VRIES, P., HEILIGERS, J.P.C., VILLALÓN, C.M. & SAXENA, P.R. (1999). Pharmacological evidence that α_1- and α_2-adrenoceptors mediate vasoconstriction of carotid arteriovenous anastomoses in anaesthetized pigs. *Br. J. Pharmacol.,* **127,** 1263 – 1271.

WYSS, J.M., SWANSON, L.W. & COWAN, M.W. (1979). A study of subcortical afferents to the hippocampal formation in the rat. *Neuroscience,* **4,** 463 – 476.

YABASE, M., CARINO, M.A. & HORITA, A. (1990). Cocaine produces cholinergically mediated analeptic and EEG arousal effects in rabbits and rats. *Pharmacol. Biochem. Behav.,* **37,** 375 – 377.

YANG, C.C.H., KUO, T.B.J. & CHAN, S.H.H. (1996). Auto- and cross-spectral analysis of cardiovascular fluctuation during pentobarbital anesthesia in the rat. *Am. J. Physiol.,* **270,** H575 – H582.

YASUDA, R.P., ZAHNISER, N.R. & DUNWIDDIE, T.V. (1984). Electrophysiological effects of cocaine in the rat hippocampus in vitro. *Neurosci. Lett.,* **45,** 199 – 204.

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