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Nitric oxide as a mediator of cocaine-induced penile erection in the rat

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1 The effect of local application of cocaine to the corpus cavernosum on intracavernous pressure (ICP), an experimental index for penile erection, was examined in Sprague-Dawley rats anaesthetized with chloral hydrate. The potential involvement of dopamine, noradrenaline or nitric oxide as the chemical mediator in this process, and the pharmacological action of cocaine as a local anaesthetic in the induced increase in ICP, were also investigated.

2 Intracavernous (i.c.) administration of cocaine (40, 80 or 160 μg) to the corpus cavernosum resulted in a dose-related increase in both amplitude and duration of ICP.

3 The elevation in ICP induced by cocaine (160 μg, i.c.) was not significantly influenced by prior injection into the corpus cavernosum of either the D_1 or D_2 dopamine receptor antagonist, **R**-(+)-SCH 23390 (250 pmol) or (−)-sulpiride (250 pmol).

4 Similarly, penile erection promoted by cocaine (160 μg, i.c.) was not appreciably affected by i.c. pretreatment with the α_1-, α_2-, or β-adrenoceptor antagonist, prazosin (50 pmol), yohimbine (50 pmol) or propranolol (5 nmol).

5 Whereas lignocaine (4 μmol, i.c.) depressed penile erection induced by papaverine (400 μg, i.c.), local application of cocaine (160 μg) into the corpus cavernosum still elicited significant elevation in ICP in the presence of lignocaine or papaverine.

6 The increase in ICP induced by cocaine (160 μg, i.c.) was attenuated dose-dependently by prior cavernosal administration of the NO synthase inhibitor, N^{ω}-nitro-L-arginine methyl ester (L-NAME, 0.5, 1 or 5 pmol) or NG-monomethyl-L-arginine (L-NMMA, 2.5, 5 or 10 pmol). The blunting effect of L-NAME or L-NMMA was reversed by co-administration of the NO precursor, L-arginine (1 nmol, i.c.).

7 Pretreatment by local application into the corpus cavernosum of methylene blue (2.5 μmol), an inhibitor of cytosolic guanylyl cyclase, antagonized cocaine-induced penile erection.

8 Direct i.c. administration of a NO donor, nitroglycerin (10 or 20 nmol), mimicked the local action of cocaine by promoting a significant increase in ICP.

9 It is concluded that cocaine may induce penile erection by increasing ICP via a local action on the corpus cavernosum. This process did not appear to involve either dopamine or noradrenaline as the chemical mediator, nor the pharmacological action of cocaine as a local anaesthetic. On the other hand, it is likely that initiation and maintenance of penile erection elicited by cavernosal application of cocaine engaged an active participation of NO and subsequent activation of guanylyl cyclase in the corpus cavernosum.

Keywords: Cocaine; corpus cavernosum; intracavernous pressure; penile erection in rat; nitric oxide

Introduction

Cocaine is an alkaloid that was isolated from *Erythroxylum coca* by Gaedcke in 1855, chemically defined by Nieman in 1859, synthesized by Willstatter in 1923, and the stereo-structure determined by von Hardeggar & Ott in 1995 (see Johanson & Fischman, 1989). In terms of pharmacological actions, cocaine is known to be a local anaesthetic (Bedford *et al.*, 1984) and a central nervous stimulant (Van Dyke & Byck, 1977; Commissaris, 1989). The former action arises from its blockade of sodium channels on the neuronal membrane (Matthews & Collins, 1983). An overflow of catecholamines at the synaptic cleft because of a blockade of their re-uptake by cocaine is generally attributed to the latter action (Hernandez *et al.*, 1988; Izenwasser *et al.*, 1990; Luoh *et al.*, 1994; Chang *et al.*, 1995).

In addition to its central effects, cocaine possesses aphrodisiac actions that include increase in libido, elicitation of penile erection, prolongation in intercourse and production of intense orgasms (Cohen, 1975). Whereas low-dose cocaine increases sexual activity, sustained use, particularly at high doses, of cocaine results in a decrease in penile erection or detumescence (Cregler & Mark, 1986). Rodriguez-Blazquez *et al.* (1990) reported that local application of cocaine to the penis induces priapism, a condition of prolonged erection not related to sexual arousal or desire. This condition was also observed in intranasal cocaine abusers (Fiorelli *et al.*, 1990). Kim *et al.* (1992), on the other hand, failed to identify a significant influence on erectile function in cocaine-dependent patients. In male rats, sexual behaviour is not significantly altered following chronic cocaine treatment (Abel *et al.*, 1989). The relationship between cocaine and penile erection is, therefore, at best, equivocal.

In human subjects and rats, it is generally believed that penile erection is mediated by opposing inputs from the parasympathetic and sympathetic nervous system to penile vascular smooth muscles via the action of acetylcholine and noradrenaline (Carati *et al.*, 1987; de Groat & Steers, 1988; Lue & Tanagho, 1988). Whereas adrenergic neurotransmission mediates contraction of corporal smooth muscles and causes detumescence, cholinergic neurotransmission promotes smooth muscle relaxation and penile erection. Recent studies

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(Juenemann *et al.*, 1987; Steers, 1990; Azadzoi *et al.*, 1992; Burnett *et al.*, 1992; Kirkeby *et al.*, 1992; Stief *et al.*, 1993) further revealed the role of non-adrenergic, non-cholinergic transmitters, including neuropeptides (e.g. vasoactive intestinal polypeptide, neuropeptide Y, somatostatin, calcitonin gene-related peptide) and nitric oxide (NO), as mediators of penile erection. The involvement of NO (Palmer *et al.*, 1987), in acetylcholine-induced penile erection is also suggested (Ignarro *et al.*, 1990; Ignarro, 1992).

Our laboratory has recently established a rat model (Chen *et al.*, 1992a), which uses the intracavernous pressure (ICP) in anaesthetized animals as the index for a more sensitive, objective and quantitative assessment of penile erection. Utilizing this experimental index, the present study was undertaken to examine the effect of direct local administration of cocaine to the corpus cavernosum on penile erection. We also evaluated the potential involvement of dopamine, noradrenaline or NO as the chemical mediator in this process. Whether its action as a local anaesthetic accounts for cocaine-induced penile erection was also investigated.

Methods

Animal preparation

Male, adult Sprague-Dawley rats (200–250 g) anaesthetized with chloral hydrate (400 mg kg^{-1}, i.p., with 40 mg kg^{-1} h^{-1}, i.v. supplements) were used. Routine surgical preparations included cannulation of the left femoral artery and vein for the measurement of systemic arterial pressure and administration of drugs. The right femoral vein was also cannulated for continuous infusion of supplemental anaesthetic. Systemic arterial pressure was monitored via a pressure transducer (Gould 23ID) and a pressure processor amplifier (Gould 13-4615-52), and heart rate was determined with a biotachometer amplifier (Gould 20-4615-65) triggered by the arterial pressure pulses. The trachea was intubated to maintain its patency and to facilitate ventilation with a rodent respirator (Harvard 683). Pulsatile and mean systemic arterial pressure (SAP and MSAP), as well as heart rate (HR), were recorded simultaneously on a polygraph (Gould ES1000). All data were collected from animals with a maintained rectal temperature of 37°C and a steady MSAP above 75 mmHg throughout the experiment.

Recording of intracavernous pressure

ICP, which was used as the experimental index for the evaluation of penile erection, was measured as described previously (Chen *et al.*, 1992a,b). In brief, the skin overlying the penis was incised, and the prepuce was degloved to expose fully both corpora cavernosa. A 26-gauge needle, connected to a pressure transducer (Gould 23ID) via a PE-20 tubing filled with saline, was carefully inserted into the corpus cavernosum on one side to measure the ICP. ICP was monitored alongside SAP, MSAP and HR during the experiment.

Intracavernous administration of chemicals

Cocaine and all test agents were administered directly into the corpus cavernosum via the ICP recording needle. Measurement of ICP was momentarily suspended during this intracavernous (i.c.) application of chemical agents, by turning off the three-way connector to the pressure transducer to allow for inflow of solution into the corpus cavernosum.

A controlled volume of 250 μl was delivered for each injection, lasting usually less than 20 s. To ensure that the ICP needle was properly lodged into the corpus cavernosum, i.c., administration of this same volume of saline was routinely executed at the beginning of the experiment to ascertain the lack of leakage of injection solution. This procedure also ascertained that the introduction of fluid directly into the corpus cavernosum may not itself be a confounding factor.

Experimental protocols

To establish the effect of cocaine on penile erection, local administration of cocaine (40, 80 or 160 μg) or saline (250 μl) to the corpus cavernosum was carried out in our first series of experiment. The elicited effects on both amplitude and duration of ICP, as well as MSAP and HR, were observed for 60 min postinjection.

Cocaine-induced central stimulation is generally attributed to an overflow of dopamine and noradrenaline at the synaptic cleft because of a blockade of their re-uptake by cocaine (Hernandez *et al.*, 1988; Izenwasser *et al.*, 1990; Luoh *et al.*, 1994; Chang *et al.*, 1995). Thus, we evaluated in our second series of experiment the possible participation of dopamine or noradrenaline as a chemical mediator in cocaine-induced penile erection. For this purpose, a dopamine or noradrenaline antagonist was applied intracavernously 30 min before the administration of cocaine (160 μg, i.c.). The effects of cocaine on ICP, as well as MSAP and HR, were followed for 60 min. The antagonists used for such pretreatments included: D_1 or D_2 dopamine receptor antagonist (Kropf *et al.*, 1989), **R**-(+)-SCH 23390 (250 pmol) or (−)-sulpiride (250 pmol); and α_1-, α_2- or β-adrenoceptor antagonist, prazosin (Massingham *et al.*, 1981; 50 pmol), yohimbine (Rouot *et al.*, 1982; 50 pmol) or propranolol (Black & Stephenson, 1962; 5 nmol).

Our third series of experiments examined the role of the well known local anaesthetic action of cocaine (Bedford *et al.*, 1984) in its elicitation of penile erection. We evaluated the response of ICP to i.c. application of lignocaine (4 μmol), given alone or 30 min before cocaine (160 μg, i.c.). In a separate group of animals, the effect of lignocaine (4 μmol, i.c.) or cocaine (160 μg, i.c.) on penile erection induced by papaverine (400 μg, i.c.), a vasoactive agent most commonly used in clinical management of impotence (Lue & Tanagho, 1987), was evaluated. Lignocaine or cocaine was administered into the corpus cavernosum when the papaverine-elicited increase in ICP reached its peak.

The role of NO in cocaine-induced penile erection was investigated in our last series of experiments. The effects of pretreatment with i.c. administration of the NO synthase inhibitor (Moncada *et al.*, 1991), N^{ω}-nitro-L-arginine methyl ester (L-NAME, 0.5, 1 or 5 pmol) or NG-monomethyl-L-arginine (L-NMMA, 2.5, 5 or 10 pmol); or the cytosolic guanylyl cyclase inhibitor (Kim *et al.*, 1991), methylene blue (2.5 μmol), on cocaine-elicited elevation in ICP were investigated. The specificity of the NO synthase inhibitors in influencing cocaine-induced increase in ICP was verified by co-injection into the corpus cavernosum of either a NO synthase inhibitor with a NO precursor (Moncada *et al.*, 1991), L-arginine (1 nmol). Intracavernous application of a NO donor (Bush *et al.*, 1992), nitroglycerin (10 or 20 nmol), was used as a positive control.

At the end of each experiment, papaverine (400 μg, i.c.) was delivered to ensure that the ICP recording needle was properly lodged into the corpus cavernosum throughout the experiment.

Drugs

Cocaine HCl was obtained from the Department of Health (Taiwan). Dopamine HCl, (−)-sulpiride HCl, noradrenaline HCl, prazosin HCl, yohimbine HCl, propranolol HCl, L-arginine, L-NAME, methylene blue, nitroglycerin and papaverine were obtained from Sigma (St. Louis, MO, U.S.A.). **R**-(+)-SCH 23390 and L-NMMA were obtained from RBI (St. Natick, MA, U.S.A.). All substances were dissolved in saline, and were freshly prepared immediately before each experiment.

Statistical evaluation

All values in the figures and text are expressed as mean ± s.e.mean. The differences between treatment groups were statistically assessed by one-way or two-way analysis of variance (ANOVA) with repeated measures, followed by Scheffe or Dunnett multiple range tests for a posteriori comparison of

means. A value at $P<0.05$ was considered to be statistically significant.

Results

Intracavernous administration of cocaine increased ICP

Figure 1 is a polygraph tracing showing the differential effect of saline and cocaine on ICP. Control i.c. injection of saline, at a volume of 250 μl, caused a transient increase in the ICP that returned to baseline within 3 min postinjection (Figure 1). In contrast, ocal application of cocaine (160 μg) to the corpus cavernosum, in the same injection volume, elicited a prolonged elevation in ICP.

The increase in both amplitude and duration of ICP by i.c. administration of cocaine (40, 80 or 160 μg) was manifested in a dose-dependent manner (Figure 2). As we previously reported (Chen *et al.*, 1992b), visible penile erection (straightening of penis with disappearance of the angle between the glans and shaft) was observed upon an elevation of ICP above the threshold pressure of 40 mmHg. On the other hand, relative to control administration of saline, changes in MSAP (73.6±2.6, 73.3±3.5 or 72.6±3.3 vs 75.6±2.9 mmHg, $n=5-8$) and HR (383±31, 382±28 or 390±18 vs. 371±17 beats min^{-1}, $n=5-8$) caused by cocaine were indiscernible for the three doses studied.

Evidence that dopaminergic neurotransmission at the corpus cavernosum does not participate in cocaine-induced elevation in ICP

Local injection into the corpus cavernosum of either D_1 or D_2 dopamine receptor antagonist, **R**-(+)-SCH 23390 (250 pmol) or (−)-sulpiride (250 pmol), did not itself significantly alter basal ICP (6.8±1.3 vs. 4.9±1.6 mmHg, $n=6$; or 5.3±0.8 vs. 6.0±0.8 mmHg, $n=5$). Such pretreatment (Figure 3) had no appreciable effect on the prolonged elevation in ICP induced by cocaine (160 μg, i.c.). Pretreatment with these dopamine receptor antagonists, on the other hand, appreciably ($P<0.05$) attenuated the rise in ICP (+25.4±2.7 mmHg, $n=5$) following local application of dopamine (2.5 μg) to the corpus cavernosum: **R**-(+)-SCH 23390 pretreatment, +1.8±1.6 mmHg, $n=6$; (−)-sulpiride pretreatment, +5.6±3.4 mmHg, $n=6$.

Evidence that noradrenergic neurotransmission at the corpus cavernosum does not participate in cocaine-induced elevation in ICP

Similar to the dopamine receptor blockers, i.c. administration of α_1-, α_2- or β-adrenoceptor antagonist, prazosin (50 pmol), yohimbine (50 pmol) or propranolol (5 nmol), did not change the basal ICP (5.1±1.1 vs. 5.9±0.7 mmHg, $n=7$; 4.7±1.3 vs. 5.2±0.4 mmHg, $n=5$; or 5.8±2.3 vs. 6.2±1.6 mmHg, $n=6$).

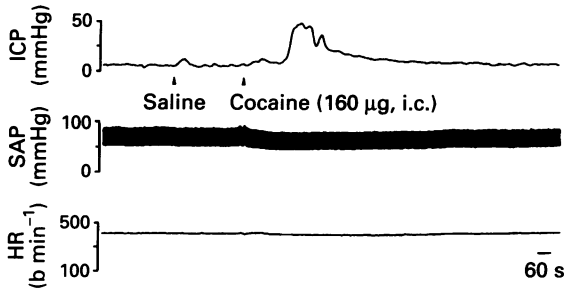

Figure 1 Representative polygraph tracing showing the time-course changes in intracavernous pressure (ICP), systemic arterial pressure (SAP) and heart rate (HR) following intracavernous (i.c.) administration of saline (250 μl) or cocaine (160 μg, 250 μl).

Relative to control administration of saline, the same pretreatments also produced no discernible effect on the amplitude (34.8±5.3, 33.4±5.9 or 27.1±5.3 vs. 32.3±3.3 mmHg, $n=5-7$) or duration (901±205, 793±211 or 1371±263 vs. 1041±140 s, $n=5-7$) of the elevation in ICP induced by cocaine (160 μg, i.c.). Pretreatment with these adrenoceptor antagonists, however, significantly ($P<0.05$) blunted the decrease in ICP (−6.1±0.8 mmHg, $n=7$) induced by noradrenaline (0.4 μg, i.c.): prazosin pretreatment, +1.3±0.9 mmHg, $n=5$; yohimbine pretreatment, −0.9±1.4 mmHg, $n=6$; propranolol pretreatment, +2.4±0.6 mmHg, $n=6$.

Evidence that local anaesthetic action does not contribute to cocaine-induced elevation in ICP

In contrast to cocaine-induced penile erection, i.c. application of lignocaine (4 μmol) caused a nominal decrease in ICP (Figure 4) and no appreciable effect on MSAP (81.3±5.5 vs. 79.0±3.2 mmHg, $n=6$) or HR (378±20 vs. 370±14 beats min^{-1}, $n=6$). However, the ability of cocaine (160 μg, i.c.) to increase ICP was not significantly affected by prior application of lignocaine (4 μmol) to the corpus cavernosum.

To ascertain further that its local anaesthetic action did not contribute to cocaine-induced penile erection, we evaluated (Figure 5) the effect of lignocaine (4 μmol, i.c.) or cocaine (160 μg, i.c.) on the elevation in ICP caused by papaverine (400 μg, i.c.). Given during the peak action of papaverine, the sustained increase in ICP was significantly reduced by i.c. administration of lignocaine (4 μmol). Cocaine (160 μg, i.c.), on the other hand, caused a further rise in ICP.

Nitric oxide at the corpus cavernosum may mediate cocaine-induced elevation in ICP

Direct application of L-NAME (0.5, 1 or 5 pmol) to the corpus cavernosum dose-dependently attenuated the cocaine-induced increase in both amplitude and duration of ICP (Figure 6). This suppressive effect of L-NAME (5 pmol, i.c.) was reversed upon concurrent administration of a NO precursor, L-arginine (1 nmol, i.c.) (Figure 6). Relative to control administration of saline, i.c. application of L-NMMA (2.5, 5 or 10 pmol) also dose-dependently reduced the amplitude (15.9±7.3, 13.4±5.8

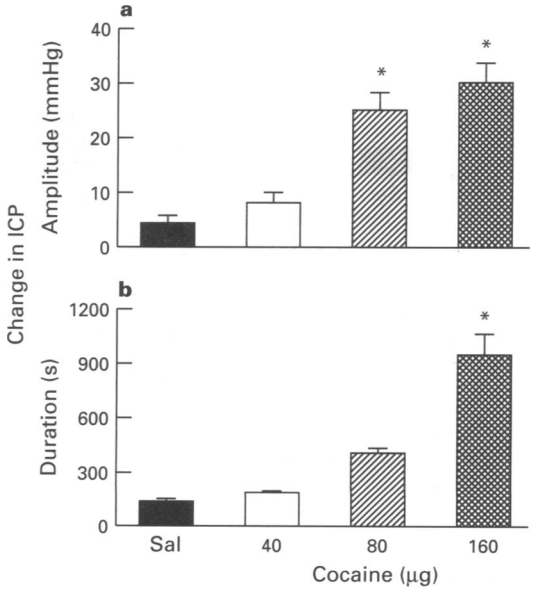

Figure 2 Maximal changes in amplitude and duration of ICP in response to i.c. administration of saline (Sal, 250 μl) or cocaine (40, 80 or 160 μg, 250 μl). Values are presented as mean±s.e.mean, $n=5-8$ animals per group. Significant difference ($P<0.05$) exists between treatment groups by one-way ANOVA analysis. *$P<0.05$ vs. saline group by the Scheffe multiple range test.

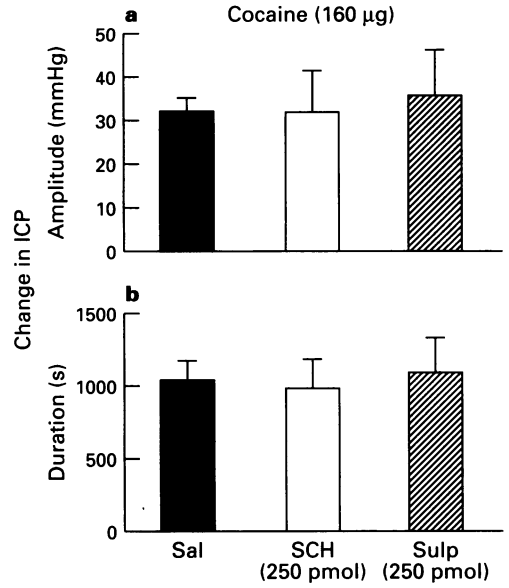

Figure 3 Maximal changes in amplitude and duration of ICP in response to i.c. administration of cocaine (160 μg), 30 min following pretreatment with i.c. application of saline (Sal, 250 μl), **R**-(+)-SCH 23390 (SCH, 250 pmol) or (−)-sulpiride (Sulp, 250 pmol). Values are presented as mean ± s.e.mean, $n = 5-7$ animals per group. No significant difference ($P < 0.05$) exists between treatment groups by one-way ANOVA analysis.

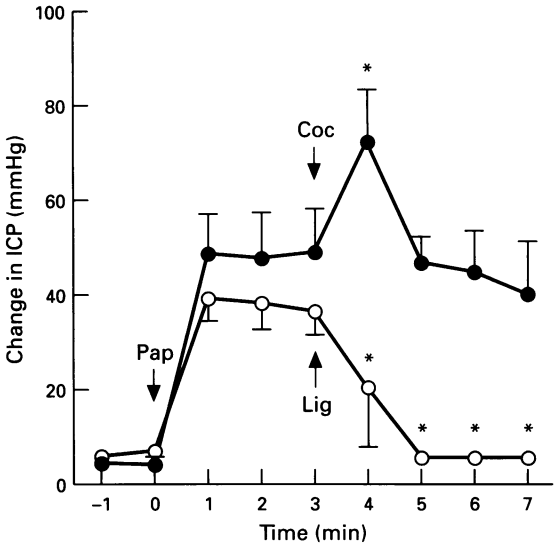

Figure 5 Time-course changes in amplitude of ICP after i.c. administration of papaverine (Pap, 400 μg), followed by cocaine (Coc, 160 μg) or lignocaine (Lig, 4 μmol) after peak effect of papaverine was reached. Values are presented as mean ± s.e.mean, $n = 5-6$ animals per group. Significant difference ($P < 0.05$) exists between treatment groups by two-way ANOVA analysis with repeated measures. *$P < 0.05$ vs. peak effect of papaverine (at 3 min) by the Dunnett multiple range test.

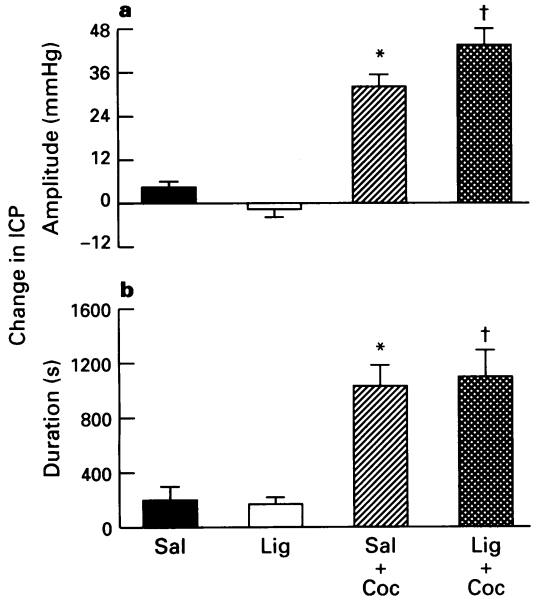

Figure 4 Maximal changes in amplitude and duration of ICP in response to i.c. administration of saline (Sal, 250 μl) or lignocaine (Lig, 4 μmol), given alone or as a pretreatment 30 min before i.c. administration of cocaine (Coc, 160 μg). Values are presented as mean ± s.e.mean, $n = 5-6$ animals per group. Significant difference ($P < 0.05$) exists between treatment groups by one-way ANOVA analysis. *$P < 0.05$ vs. saline group and †$P < 0.05$ vs. lignocaine group by the Scheffe multiple range test.

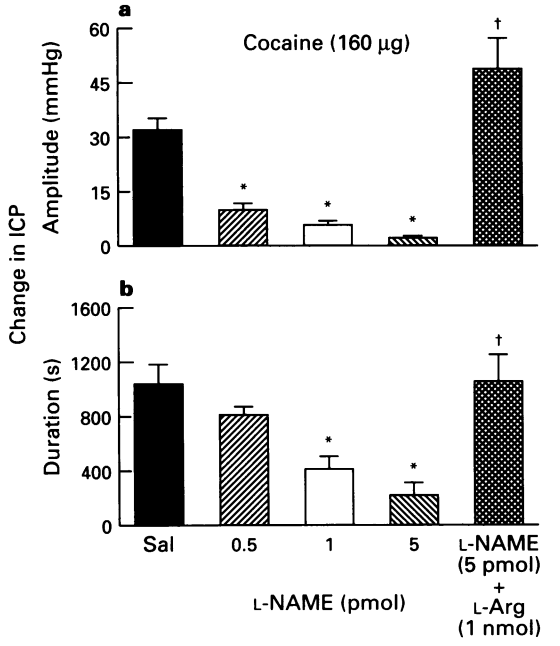

Figure 6 Maximal changes in amplitude and duration of ICP in response to i.c. administration of cocaine (160 μg), 30 min following pretreatment with i.c. injection of saline (Sal, 250 μl), N^{ω}-nitro-L-arginine methyl ester (L-NAME, 0.5, 1 or 5 pmol) or L-NAME and L-arginine (L-Arg, 5 pmol and 1 nmol). Values are presented as mean ± s.e.mean, $n = 5-7$ animals per group. Significant difference ($P < 0.05$) exists between treatment groups by one-way ANOVA analysis. *$P < 0.05$ vs. saline group and †$P < 0.05$ vs. L-NAME (5 pmol) group by the Scheffe multiple range test.

or 11.5 ± 4.9 vs. 32.2 ± 3.3 mmHg, $n = 6-8$) and shortened the duration (990 ± 246, 438 ± 136 or 414 ± 92 vs. 1041 ± 140 s, $n = 6-8$) of the elevation in ICP by cocaine (160 μg, i.c.). Likewise, co-administration of L-NMMA and L-arginine (10 pmol + 1 nmol, i.c.) reversed the antagonist action of L-NMMA. Pretreatment with either NO synthase inhibitor, alone or in combination with L-arginine, on the other hand, exerted no significant effect on basal ICP (Table 1).

The involvement of NO in cocaine-induced penile erection was further demonstrated when pretreatment with guanylyl cyclase inhibitor, methylene blue (2.5 μmol, i.c.), significantly ($P < 0.05$) suppressed the cocaine-induced increase in ICP

(9.6±4.7 vs. 32.3±3.3 mmHg; 369±20 vs. 1041±140 s; $n=5-6$). Furthermore, relative to saline, direct application of nitroglycerin (10 or 20 nmol) into the corpus cavernosum also increased (25.1±4.4 or 53.7±3.1 vs. 4.6±1.2 mmHg, $n=5-7$) and prolonged (190±55 or 296±50 vs. 141±11 s, $n=5-7$) the ICP.

Discussion

The present study demonstrates that direct application of cocaine to the corpus cavernosum of the penis increases and prolongs ICP in the rat. Our data further reveal that such an erectile effect of cocaine does not appear to be related to its well established pharmacological actions, namely, modulation of dopaminergic or noradrenergic neurotransmission and blockade of sodium channels as a local anaesthetic. Instead, it is likely that elevation in ICP by cocaine may be mediated by NO at the corpus cavernosum.

Our study was greatly facilitated by the experimental index we used for penile erection (Chen *et al.*, 1992a, b). Although i.c. administration of cocaine at 160 μg elicited a five fold increase in ICP from baseline, the magnitude was in many cases still below 40 mmHg, the threshold pressure for visible penile erection. This superb detection sensitivity therefore allowed us to measure the erectile effect of cocaine that was otherwise undetectable by behavioural assessments.

Sexual dysfunction is one of the factors that motivates patients to seek treatment for cocaine abuse (Cregler & Mark, 1986). Unfortunately, the relationship between cocaine and male sexual behaviour is still controversial. Our demonstration of prolonged increase in ICP by direct application of cocaine to the corpus cavernosum of rodents was in agreement with previous clinical reports that associate priapism with intraurethral (Mahler *et al.*, 1988) or topical (Rodriguez-Blazquez *et al.*, 1990) administration of cocaine. Our observations, however, are at variance with a recent demonstration (Pomerantz *et al.*, 1994) of an impairment of male copulatory behaviour in rhesus monkeys following acute intramuscular administration of cocaine. Such a discrepancy may be due to the difference in species of animal used, route of administration and dose of cocaine.

Cocaine exerts its pharmacological effects via both central and peripheral mechanisms. The central stimulatory action of cocaine results from its inhibition on the re-uptake of dopamine, noradrenaline and 5-hydroxytryptamine at the synaptic cleft (Taylor & Ho, 1978; Hadfield *et al.*, 1980; Bagchi & Reilly, 1983; Luoh *et al.*, 1994; Chang *et al.*, 1995). Cocaine also facilitates the release of dopamine from reserpine-sensitive storage pools (Heikkila *et al.*, 1975) and stimulates tyrosine hydroxylase activity (Taylor & Ho, 1978; Akbari & Azmitia, 1992). By inhibiting the re-uptake of noradrenaline at the neuroeffector junction, the major peripheral effect of cocaine appears to be sympathomimetic (Van Dyke & Byck, 1982; Jain *et al.*, 1990; Tella *et al.*, 1990; Schindler *et al.*, 1992). Thus, it is intriguing that at a dose that significantly antagonized the respective increase and decrease in ICP by dopamine and noradrenaline, local application into the corpus cavernosum of D_1 or D_2 dopamine receptor antagonists or α_1-, α_2- or β-adrenoceptor blockers elicited no appreciable effect on cocaine-induced penile erection. Thus, it appears that dopamine or noradrenaline may not function as chemical mediators in the corpus cavernosum for the prolonged increase in ICP generated by i.c. application of cocaine.

It is possible that stimulation of dopamine receptors by cocaine may be more discrete than that accomplished through direct dopamine administration (Pomerantz *et al.*, 1994). Since cocaine augments stimulation of dopamine receptors by blocking the re-uptake of the biogenic amine, it exerts its action only in those regions that are actively releasing dopamine. Thus, a direction for future investigation is to delineate the existence of dopaminergic innervation of cavernous tissue and the role of endogenous dopamine in the regulation of penile erection.

Based on its blocking effect on nerve conduction (Matthews & Collins, 1983; Bedford *et al.*, 1984), cocaine is used topically on the glans penis to decrease sensitivity and prolong erection time (Mahler *et al.*, 1988; Rodriguez-Blazquez *et al.*, 1990). Our data, nonetheless, suggest that cocaine may not share the same mechanism of action with the local anaesthetic, lignocaine, in its influence on ICP. Local application of cocaine and lignocaine to the cavernous tissue respectively enhanced and reduced basal or papaverine-induced elevation in ICP. Furthermore, prolonged increase in ICP by i.c. administration of cocaine was still observed in the presence of lignocaine.

In man and non-primate animals, the smooth muscle tone of trabecular corpus cavernosum is controlled by at least three pathways, namely, adrenergic, cholinergic and non-adrenergic, non-cholinergic axon innervations. Noradrenaline causes detumescence by contracting corporal smooth muscle, and acetylcholine promotes smooth muscle relaxation and penile erection (Saenz de Tejada *et al.*, 1988; Keast, 1992; 1995; Burnett *et al.*, 1992; Rajfer *et al.*, 1992; Brock & Lue, 1993). During the past decade, NO has gained interest as the physiological mediator of non-adrenergic, non-cholinergic neurotransmission in penile erection. The enzyme responsible for the formation of NO, NO synthase, is localized in neurones that innervate the penile smooth muscle (Keast, 1992; Burnett *et al.*, 1993; Ding *et al.*, 1993; Vizzard *et al.*, 1994). Direct application of the precursor or donor for NO relaxes the corpus cavernosum in a manner similar to the relaxation produced by pelvic nerve stimulation (Ignarro *et al.*, 1990; Kim *et al.*, 1991; Pickard *et al.*, 1991; Bush *et al.*, 1992; Ignarro, 1992; Rajfer *et al.*, 1992; Trigo-Rocha *et al.*, 1993b; Wang *et al.*, 1994). Furthermore, neurally activated tumescence is inhibited by a blockade of NO synthesis or the signal transduction cascade of NO (Burnett *et al.*, 1992; Trigo-Rocha *et al.*, 1993b; Hull *et al.*, 1994). On the other hand, prolonging the activity of cyclic

Table 1 Effect on the amplitude of ICP, 30 min after intracavernous administration of a NO synthase inhibitor, given alone or in combination with L-arginine

Treatment		*ICP*	
		Control	*Post-treatment*
Saline	(250 μl)	5.2±0.8	5.9±0.8
L-NAME	(0.5 pmol)	6.7±1.8	7.1±1.7
L-NAME	(1 pmol)	7.0±1.7	7.5±0.5
L-NAME	(5 pmol)	5.8±0.7	6.2±1.0
L-NAME + L-Arg	(5 pmol + 1 nmol)	6.4±1.5	6.0±1.6
L-NMMA	(2.5 pmol)	5.4±0.9	5.6±0.4
L-NMMA	(5 pmol)	6.3±1.2	6.0±0.9
L-NMMA	(10 pmol)	5.0±0.8	4.8±0.5
L-NMMA + L-Arg	(10 pmol + 1 nmol)	5.0±1.6	5.1±1.7

Mean±s.e.mean, n = 5–8 animals per group.

guanosine monophosphate, the second messenger activated by NO (Arnold *et al.*, 1977) to cause relaxation of corporal smooth muscle (Ignarro *et al.*, 1990), enhances pelvic nerve-stimulated penile erection (Trigo-Rocha *et al.*, 1993a).

Our present results provide an additional functional role for NO in erectile functions by demonstrating its participation as a mediator in the corpus cavernosum for cocaine-induced penile erection. We found that the prolonged increase in ICP by i.c. adminstration of cocaine was antagonized by two inhibitors of NO synthase and an inhibitor of cytosolic guanylyl cyclase. The former antagonism was reversible by a precursor of NO. In addition, direct application of a NO donor into the corpus cavernosum mimicked the action of cocaine and promoted a significant increase in ICP.

The source of NO in the corpus cavernosum that participates in cocaine-induced penile erection is not readily available from the present data. There are at least two possible stores of NO within the penis. One is the endothelium of penile blood vessels (de Groat & Steers, 1988), the other is neurones in the major pelvic ganglia (Keast, 1992; Ding *et al.*, 1993) which send the NO synthase-containing cavernous nerve to innervate the penile erectile tissue. The involvement of either or both sources in cocaine-induced elevation in ICP must await further elucidation. Since blockade of NO synthesis or interruption of the signal transduction cascade of NO produced no discernible influence on basal ICP, it is likely that the constitutive pool of NO may not play a regulatory role in the maintenance of smooth muscle tone in the trabecular corpus cavernosum.

Rodriguez-Blazquez *et al.* (1990) proposed that prolongation of penile erection in cocaine-related priapism is the result of impairment of the detumescence process due to local depletion of noradrenaline, produced by an increased exposure of this neurotransmitter to degradative enzymes at the neuroeffector junctions of cavernous smooth muscle. Our present data suggest, on the other hand, that a maintained level of NO is crucial to the prolonged erectile response to cocaine. The duration of increase in ICP by local application of cocaine in the presence of NO synthase inhibitors was similar to that of i.c. application of saline. We also found that addition of a NO precursor reversed the antagonism of NO synthase inhibitors on cocaine-induced elevation in ICP to an amplitude and duration that were comparable to that elicited by vehicle control. These observations imply that NO plays an important role in both the initiation and maintenance of tumescence following cocaine administration.

One of the side effects of cocaine at the periphery is the production of hypertension and tachycardia (Wilkerson, 1988; Tella *et al.*, 1990; Schindler *et al.*, 1992). Thus, it is possible that our observed cocaine-induced penile erection may be secondary to its action on the systemic circulation, which alters arterial inflow to and venous outflow from corporal sinusoids. This possibility, however, is considered unlikely, since local administration of cocaine into the corpus cavernosum did not appreciably influence SAP and HR. Nonetheless, whether cocaine influences local circulation at the penile tissue awaits further investigation.

In conclusion, our data reveal, for the first time, that direct administration of cocaine to the corpus cavernosum of rats produces a prolonged increase in ICP. We further demonstrate that NO may serve as a mediator in this erectile action of cocaine. These findings may be of significance for new therapeutic approaches to the treatment of erectile dysfunctions associated with cocaine abuse.

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References

ABEL, E.L., MOORE, C., WASELEWSKY, D., ZAJAC, C. & RUSSELL, L.D. (1989). Effects of cocaine hydrochloride on reproductive function and sexual behavior of male rats and on the behavior of their offspring. *J. Androl.*, **10**, 17–27.

AKBARI, H.M. & AZMITIA, E.C. (1992). Increased tyrosine hydroxylase immunoreactivity in the rat cortex following prenatal cocaine exposure. *Dev. Brain Res.*, **66**, 277–281.

ARNOLD, W.P., MITTAL, C.K., KATSUKI, S. & MURAD, F. (1977). Nitric oxide activates guanylate cyclase and increases guanosine 3',5'-cyclic monophosphate levels in various tissue preparations. *Proc. Natl. Acad. Sci. U.S.A.*, **74**, 3203–3207.

AZADZOI, K.M., KIM, N., BROWN, M.L., GOLDSTEIN, I., COHEN, R.A. & SAENZ DE TEJADA, I. (1992). Endothelium-derived nitric oxide and cyclooxygenase productions modulate corpus cavernosum smooth muscle tone. *J. Urol.*, **147**, 220–225.

BAGCHI, S.P. & REILLY, M.A. (1983). Intraneural dopaminergic action of cocaine and some of its metabolites and analogs. *Neuropharmacology*, **22**, 1289–1295.

BEDFORD, J.A., TURNER, C.E. & ELSOHLY, H.N. (1984). Local anesthetic effects of cocaine and several extracts of the coca leaf (*E. coca*). *Pharmacol. Biochem. Behav.* **20**, 819–821.

BLACK, J.W. & STEPHENSON, J.S. (1962). Pharmacology of a new *beta*-adrenergic receptor blocking compound. *Lancet*, **ii**, 311–314.

BROCK, G.B. & LUE, T.F. (1993). Drug-induced male sexual dysfunction. *Drug Safety*, **8**, 414–426.

BURNETT, A.L., LOWENSTEIN, C.J., BREDT, D.S., CHANG, T.S.K. & SNYDER, S.H. (1992). Nitric oxide: a physiological mediator of penile erection. *Science*, **257**, 401–403.

BURNETT, A.L., TILLMAN, S.L., CHANG, T.S.K., EPSTEIN, J.I., LOWENSTEIN, C.J., BREDT, D.S., SNYDER, S.H. & WALSH, P.C. (1993). Immunohistochemical localization of nitric oxide synthase in the autonomic innervation of the human penis. *J. Urol.*, **150**, 73–76.

BUSH, P.A., ARONSON, W.J., BUGA, G.M., RAJFER, J. & IGNARRO, L.J. (1992). Nitric oxide is a potent relaxant of human and rabbit corpus cavernosum. *J. Urol.*, **147**, 1650–1655.

CARATI, C.J., CREED, K.E. & KEOGH, E.J. (1987). Autonomic control of penile erection in the dog. *J. Physiol.*, **384**, 525–538.

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 rats: 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. (1992a). Intracavernous pressure as an experimental index in a rat model for the evaluation of penile erection. *J. Urol.*, **147**, 1124–1128.

CHEN, K.-K., CHAN, J.Y.H., CHANG, L.S., CHEN, M.-T. & CHAN, S.H.H. (1992b). Elicitation of penile erection following activation of the hippocampal formation in the rat. *Neurosci. Lett.*, **141**, 218–222.

COHEN, S. (1975). Cocaine. *J. Am. Med. Ass.*, **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.

DE GROAT, W.C. & STEERS, W.D. (1988). Neuroanatomy and neurophysiology of penile erection. In *Contemporary Management of Impotence and Fertility*, ed. Tanagho, E.A., Lue, T.F. & McClure, R.D. pp.3–27, Baltimore: Williams & Wilkins.

DING, Y.-Q., WANG, Y.-Q., QIN, B.-Z. & LI, J.-S. (1993). The major pelvic ganglion is the main source of nitric oxide synthase-containing nerve fibres in penile erectile tissue of the rat. *Neurosci. Lett.*, **164**, 187–189.

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

HADFIELD, M.G., MOH, D.E.W. & ISMAY, J.A. (1980). Cocaine: effects of in vivo administration on synaptosomal uptake of norepinephrine. *Biochem. Pharmacol.*, **29,** 1861–1863.

HEIKKILA, R.E., ORLANSKY, H. & COHEN, G. (1975). Studies on the distinction between uptake inhibition and release of [^{3}H]dopamine in rat brain tissue slices. *Biochem. Pharmacol.*, **24,** 847–852.

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

HULL, E.M., LUMLEY, L.A., MATUSZEWICH, L., DOMINGUEZ, J., MOSES, J. & LORRAIN, D.S. (1994). The roles of nitric oxide in sexual function of male rats. *Neuropharmacology*, **33,** 1499–1504.

IGNARRO, L.J. (1992). Nitric oxide as the physiological mediator of penile erection. *J. NIH Res.*, **4,** 59–62.

IGNARRO, L.J., BUSH, P.A., BUGA, G.M., WOOD, K.S., FUKUTO, J.M. & RAJFER, J. (1990). Nitric oxide and cyclic GMP formation upon electrical field stimulation cause relaxation of corpus cavernosum smooth muscle. *Biochem. Biophys. Res. Commun.*, **170,** 843–850.

IZENWASSER, S., WERLING, L.L. & COX, B.M. (1990). Comparison of the effects of cocaine and other inhibitors of dopamine uptake in rat striatum, nucleus accumbens, olfactory tubercle, and medial prefrontal cortex. *Brain Res.*, **520,** 303–309.

JAIN, R.K., JAIN, M.K., BACHENHEIMER, L.C., VISNER, M.S., HAMOSH, P., TRACY, C.M. & GILLIS, R.A. (1990). Factors determining whether cocaine will potentiate the cardiac effects of neurally released norepinephrine. *J. Pharmacol. Exp. Ther.*, **252,** 147–153.

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

JUENEMANN, K.-P., LUE, T.F., LUO, J.-A., JADALLAH, S.A., NUNES, L.L. & TANAGHO, E.A. (1987). The role of vasoactive intestinal polypeptide as a neurotransmitter in canine penile erection: a combined in vivo and immunohistochemical study. *J. Urol.*, **138,** 871–877.

KEAST, J.R. (1992). A possible neural source of nitric oxide in the rat penis. *Neurosci. Lett.*, **143,** 69–73.

KEAST, J.R. (1995). Visualization and immunohistochemical characterization of sympathetic and parasympathetic neurons in the male rat major pelvic ganglion. *Neuroscience*, **66,** 655–662.

KIM, A., GALANTER, M., CASTANEDA, R., LIFSHUTZ, H. & FRANCO, H. (1992). Crack cocaine use and sexual behavior among psychiatric inpatients. *Am. J. Drug. Alc. Abuse*, **18,** 235–246.

KIM, N., AZADZOI, K.M., GOLDSTEIN, I. & SAENZ DE TEJADA, I. (1991). A nitric oxide-like factor mediates nonadrenergic-noncholinergic relaxation of penile corpus cavernosum smooth muscle. *J. Clin. Invest.*, **88,** 112–118.

KIRKEBY, H.J., FAHRENKRUG, J., HOLMQUIST, F. & OTTESEN, B. (1992). Vasoactive intestinal polypeptide (VIP) and peptide histidine methionine (PHM) in human penile corpus cavernosum tissue and circumflex veins: localization and in vitro effects. *Eur. J. Clin. Invest.*, **22,** 24–30.

KROPF, W., KUSCHINSKY, K. & KRIEGLSTEIN, J. (1989). Apomorphine-induced alterations in cortical EEG activity of rats: involvement of D-1 and D-2 dopamine receptors. *Naunyn-Schmied. Arch. Pharmacol.*, **340,** 718–725.

LUE, T.F. & TANAGHO, E.A. (1987). Physiology of erection and pharmacological management of impotence. *J. Urol.*, **137,** 829–836.

LUE, T.F. & TANAGHO, E.A. (1988). Hemodynamics of erection. In *Contemporary Management of Impotence and Infertility*. ed. Tanagho, E.A., Lue, T.F. & McClure, R.D. pp. 28–38, Baltimore: Williams & Wilkins.

LUOH, H.-F., KUO, T.B.J., CHAN, S.H.H. & PAN, W.H.T. (1994). Power spectral analysis of electroencephalographic desynchronization induced by cocaine in rats: correlation with microdialysis evaluation of dopaminergic neurotransmission at the medial prefrontal cortex. *Synapse*, **16,** 29–35.

MAHLER, J.C., PERRY, S. & SUTTON, B. (1988). Intraurethral cocaine administration. *J. Am. Med. Ass.*, **259,** 3126.

MASSINGHAM, R., DUBOCOVICH, M.L., SHEPPERSON, N.B. & LANGER, S.Z. (1981). In vivo selectivity of prazosin but not of WB-4101 for postsynaptic alpha-1 adrenoceptors. *J. Pharmacol. Exp. Ther.*, **217,** 467–474.

MATTHEWS, J.C. & COLLINS, A. (1993). Interactions of cocaine and cocaine congeners with sodium channels. *Biochem. Pharmacol.*, **32,** 455–460.

MONCADA, S., PALMER, R.M.J. & HIGGS, E.A. (1991). Nitric oxide: physiology, pathophysiology and pharmacology. *Pharmacol. Rev,*, **43,** 109–142.

PALMER, R.M.J., FERRIGE, A.G. & MONCADA, S. (1987). Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature*, **327,** 524–526.

PICKARD, R.S., POWELL, P.H. & ZAR, M.A. (1991). The effect of inhibitors of nitric oxide biosynthesis and cyclic GMP formation on nerve-evoked relaxation of human cavernosal smooth muscle. *Br. J. Pharmacol.*, **104,** 755–759.

POMERANTZ, S.M., HEPNER, B.C. & WERTZ, J.M. (1994). Impairment of male corpulatory behavior in rhesus monkeys following acute adminstration of cocaine. *Life Sci.*, **54,** 917–924.

RAJFER, J., ARONSON, W.J., BUSH, P.A., DOREY, F.J. & IGNARRO, L.J. (1992). Nitric oxide as a mediator of relaxation of the corpus cavernosum in response to nonadrenergic-noncholinergic neurotransmission. *New Engl. J. Med.*, **326,** 90–94.

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

ROUOT, B., QUENNEDEY, M.C. & SCHWARTZ, J. (1982). Characteristics of the [^{3}H]-yohimbine binding on rat brain α_2-adrenoceptors. *Naunyn-Schmied. Arch. Pharmacol.*, **321,** 253–259.

SAENZ DE TEJADA, I., BLANCO, R., GOLDSTEIN, I., AZADZOI, K., DE LAS MORENAS, A., KRANE, R.J. & COHEN, R.A. (1988). Cholinergic neurotransmission in human corpus cavernosum. I. Responses of isolated tissue. *Am. J. Physiol.*, **254,** H459–H467.

SCHINDLER, C.W., TELLA, S.R., KATZ, J.L. & GOLDBERG, S.R. (1992). Effects of cocaine and its quarternary derivative cocaine methiodide on cardiovascular function in squirrel monkeys. *Eur. J. Pharmacol.*, **213,** 99–105.

STEERS, W.D. (1990). Neural control of penile erection. *Sem. Urol.*, **8,** 66–79.

STIEF, C.G., BENARD, F., BOSCH, R., ABOSEIF, S., WETTERAUER, U., LUE, T.F. & TANAGHO, E.A. (1993). Calcitonin gene-related peptide: possibly neurotransmitter contributes to penile erection in monkeys. *Urology*, **41,** 397–401.

TAYLOR, D. & HO, B.T. (1978). Comparison of inhibition of monoamine uptake by cocaine, methylphenidate and amphetamine. *Res. Commun. Chem. Pathol. Pharmacol.*, **21,** 67–75.

TELLA, S.R., SCHINDLER, C.W. & GOLDBERG, S.R. (1990). The role of central and autonomic neural mechanisms in the cardiovascular effects of cocaine in conscious squirrel monkeys. *J. Pharmacol. Exp. Ther.*, **252,** 491–499.

TRIGO-ROCHA, F., ARONSON, W.J., HOHENFELLNER, M., IGNARRO, L.J., RAJFER, J. & LUE, T.F. (1993a). Nitric oxide and cGMP: mediators of pelvic nerve-stimulated erection in dogs. *Am. J. Physiol.*, **264,** H419–H422.

TRIGO-ROCHA, F., HSU, G.L., DONATUCCI, C.F. & LUE, T.F. (1993b). The role of cyclic adenosine monophosphate, cyclic guanosine monophosphate, endothelium and nonadrenergic, noncholinergic neurotransmission in canine penile erection. *J. Urol.*, **149,** 872–877.

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

VAN DYKE, C. & BYCK, R. (1982). Cocaine. *Sci. Am.*, **246,** 128–141.

VIZZARD, M.A., ERDMAN, S.L., FORSTERMANN, U. & DE GROAT, W.C. (1994). Differential distribution of nitric oxide synthase in neural pathways to the urogenital organs (urethra, penis, urinary bladder) of the rat. *Brain Res.*, **646,** 279–291.

WANG, R., DOMER, F.R., SIKKA, S.C., KADOWITZ, P.J. & HELLSTROM, W.J.G. (1994). Nitric oxide mediates penile erection in cats. *J. Urol.*, **151,** 234–237.

WILKERSON, R.D. (1988). Cardiovascular effects of cocaine in conscious dogs: importance of fully functional autonomic and central nervous systems. *J. Pharmacol. Exp. Ther.*, **246,** 466–471.

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