ELSEVIER

European Journal of Pharmacology 319 (1997) 71–75

ejp

Short communication

Adrenomedullin induces penile erection in the cat

Hunter C. Champion, Run Wang, Ben B. Shenassa, William A. Murphy, David H. Coy, Wayne J.G. Hellstrom, Philip J. Kadowitz *

Departments of Pharmacology and Urology, Tulane University School of Medicine, New Orleans, LA 70112, USA

Received 12 November 1996; accepted 22 November 1996

Abstract

The present study was undertaken to investigate the effects of intracavernosal injections of adrenomedullin, a novel hypotensive peptide, on penile erection in anesthetized cats. Responses to adrenomedullin were compared to those elicited by intracavernosal injection of the control triple-drug combination (1.65 mg papaverine, 25 μg phentolamine, and 0.5 μg prostaglandin E_1). Intracavernosal injections of adrenomedullin in doses of 0.1–1.0 nmol elicited dose-related increases in cavernosal pressure and penile length. The maximal effect of adrenomedullin injection on cavernosal pressure was an 8-fold increase in pressure, which was 74% of that induced by the triple-drug combination. The maximal effect on penile length was a 43% increase when compared to baseline value, which was comparable to that induced by the triple-drug combination. The duration of the peak pressure and total duration of the peptide effect were significantly shorter in response to the 1 nmol dose of adrenomedullin than was observed with the control triple-drug combination. Intracavernous injection of the control triple-drug combination resulted in a significantly greater decrease in systemic arterial blood pressure than did adrenomedullin. Erectile responses to adrenomedullin were not altered following administration of the nitric oxide synthase inhibitor, N^{ω}-nitro-L-arginine, at a time when erectile responses to acetylcholine were significantly reduced. These data demonstrate that intracavernous injection of adrenomedullin induces a short-lived erection in cats that is not due to the release of nitric oxide.

Keywords: Adrenomedullin; Penile erection; (Cat); Cavernosal pressure

1. Introduction

A number of neuropeptides and neurotransmitters have been shown to induce and modulate the erectile response at the level of the central nervous system (Bertolini and Gessa, 1981; Steers, 1990; Argiolas et al., 1993; Argiolas and Melis, 1995). Adrenocorticotropic hormone (ACTH) and melanocyte stimulating hormone (MSH) have activity at the level of the central nervous system to induce penile erection in many species (Bertolini and Gessa, 1981; Steers, 1990). Oxytocin, a neurohypophysial peptide, has been demonstrated to be a powerful inducer of penile erection with intracerebroventricular administration in the rat (Steers, 1990; Argiolas et al., 1993; Argiolas and Melis, 1995). It has recently been shown that nitric oxide mediates the erectile response induced by oxytocin (Argiolas and Melis, 1995). Nitric oxide synthase in the central nervous system has also been demonstrated to be involved in the behavioral expression of male sexual activity (Benelli et al., 1995).

Nitric oxide is believed to be involved in the erectile response at a peripheral level, as well as in the central nervous system (Steers, 1990; Burnett et al., 1992; Anderson, 1993; Wang et al., 1994). Acetylcholine released from parasympathetic neurons in the penis has been shown to produce penile erection that is mediated by the release of nitric oxide (DeGroat and Booth, 1980; Steers, 1990; Burnett et al., 1992; Anderson, 1993; Lugg et al., 1995). However, a nonadrenergic noncholinergic mechanism has also been postulated to be involved in mediating penile erection (Anderson, 1993; Wang et al., 1994; Lugg et al., 1995). Vasoactive intestinal polypeptide (VIP), which is localized in nerve fibers around cavernous smooth muscle and blood vessels, has been postulated to be a neurotransmitter and/or neuromodulator of erectile function (Steers, 1990; Anderson, 1993). Similarly, calcitonin gene-related peptide (CGRP), a potent vasodilator and smooth muscle relaxant, has recently been reported to induce penile erections in primates and may serve to modulate penile erection (Steif et al., 1993).

* Corresponding author. Department of Pharmacology, Tulane University School of Medicine, 1430 Tulane Avenue, New Orleans, LA 70112, USA. Tel.: (1-504) 88-5444; Fax: (1-504) 588-5283.

PII S0014-2999(96)00924-7

Adrenomedullin is a recently discovered hypotensive peptide isolated from human pheochromocytoma cells (Kitamura et al., 1993). Human adrenomedullin is composed of 52 amino acids with a disulfide bond that forms a six-member ring structure (Kitamura et al., 1993; Ichiki et al., 1994). This ring structure is similar to that found in CGRP and pancreatic amylin (Kitamura et al., 1993; Ichiki et al., 1994). Adrenomedullin has been identified in a number of organ systems, is present in human plasma and may serve as a circulating hormone that regulates systemic arterial pressure (Kitamura et al., 1993; Ichiki et al., 1994). Adrenomedullin decreases systemic arterial pressure and has potent vasodilator actions in the hindlimb and pulmonary vascular beds in the cat and in the systemic hindlimb and pulmonary vascular beds of the rat and the renal vascular bed of the dog (Kitamura et al., 1993; Feng et al., 1994; Champion et al., 1996; Majid et al., 1996; Nossaman et al., 1996).

Previous studies have demonstrated that the adult cat is a useful model for the study of penile erectile responses to intracavernous injections of vasoactive agents (Wang et al., 1993, 1994). However, little if anything is known about the effects of intracavernosal injection of adrenomedullin on erectile function in the feline model. The present study was, therefore, undertaken to investigate the effects of intracavernosal injection of adrenomedullin on erectile function in the cat and to investigate the role of nitric oxide release in mediating the response to intracavernosal injection of adrenomedullin. Responses to adrenomedullin were compared with responses to a standard reference (a combination of 1.65 mg papaverine, 0.5 μg prostaglandin E_1, and 25 μg phentolamine), which is commonly used in clinical practice for the treatment of impotence (Wang et al., 1993, 1994).

2. Materials and methods

Adult male cats weighing 3.5–4.8 kg were sedated with ketamine hydrochloride (10–15 mg/kg i.m.) and anesthetized with sodium pentobarbital (30 mg/kg i.v.). Supplemental doses of pentobarbital were administered as needed to maintain a uniform level of anesthesia. A vertical, circumcision-like incision was made to expose the two ventral corpora cavernosa and the dorsal corpus spongiosum. A 30-gauge needle was placed into the right corpus to permit administration of drugs into the penis. A 25-gauge needle was placed midway into the left corpus for the measurement of intracavernous pressure. Systemic arterial and intracavernous pressures (mmHg) were measured with Statham P23 transducers connected to a Grass model 7 polygraph as previously described (Wang et al., 1993). Penile length (mm) was measured with a ruler. These procedures have been approved by the Tulane University Animal Care and Use Committee.

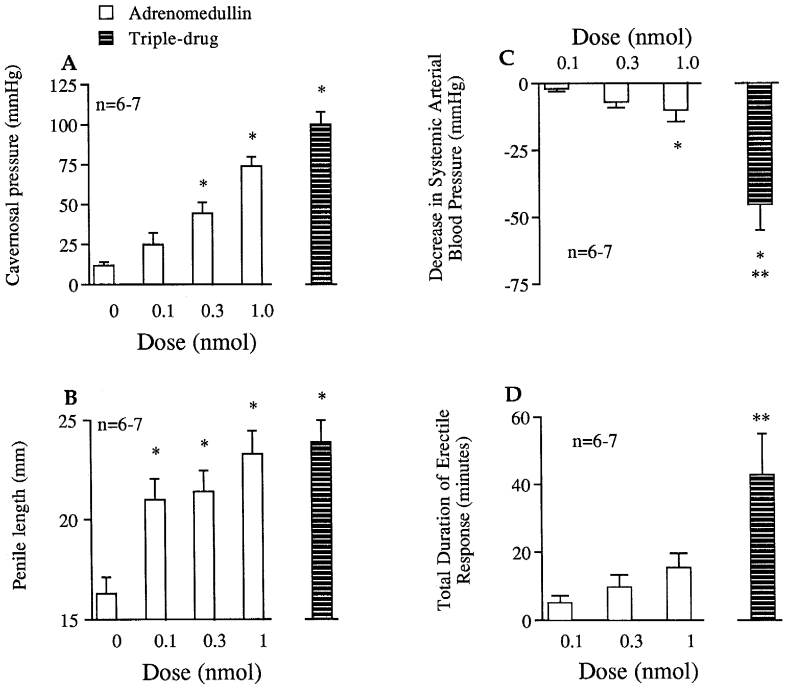

Fig. 1. (Left) Bar graphs showing dose-related increases in cavernosal pressure (A) and penile length (B) in response to intracavernosal injection of adrenomedullin and triple-drug reference response to combination of phentolamine, papaverine, and prostaglandin E_1. (Right) Bar graph showing dose-related decreases in systemic arterial pressure (C) and total duration of erectile response (in min) (D) in response to intracavernosal injection of adrenomedullin and the triple-drug combination of phentolamine, papaverine, and prostaglandin E_1. Agonists were injected directly into the corpus, and data are expressed as mean ± S.E. *n* = number of experiments. * Statistically significant from baseline value; ** statistically significant from response to 1 nmol adrenomedullin.

Synthetic human adrenomedullin (Peptide Laboratory, Tulane University Medical School, New Orleans, LA, USA) was dissolved in normal saline. The polypeptide was synthesized using standard solid-phase chemistry, and the purity was determined by reverse-phase, high-performance liquid chromatography. N^{ω}-Nitro-L-arginine (Sigma, St. Louis, MO, USA) was dissolved in acidified normal saline with sonication immediately before use. Acetylcholine chloride (Sigma) was dissolved in 0.9% NaCl. The papaverine (1.65 mg), prostaglandin E_1 (0.5 μg), and phentolamine (25 μg) (Sigma) combination was prepared and used as previously described (Wang et al., 1993, 1994).

In each animal, adrenomedullin (200 μl) was injected intracavernously in doses of 0.1, 0.3, and 1 nmol. After the dose-response curve for adrenomedullin was attained, the control combination of papaverine, prostaglandin E_1 and phentolamine was administered for comparative purposes. In studies to examine the role of nitric oxide synthesis/release in mediating responses to intracavernosal injection of adrenomedullin, the dose of adrenomedullin producing the maximal effect in the model was compared before and 30 min after intracavernosal injection of the nitric oxide synthase inhibitor, N^{ω}-nitro-L-arginine, in a dose of 20 mg. Injection of acetylcholine in a dose of 200 μg was used as an internal control to assess the level of nitric oxide synthase inhibition.

The data were expressed as mean ± S.E.M. and analyzed by Student's *t*-test for single group comparison and by one-way analysis of variance with Tukey's test for multiple-group comparisons. The value of $P < 0.05$ was established as the criterion for statistical significance.

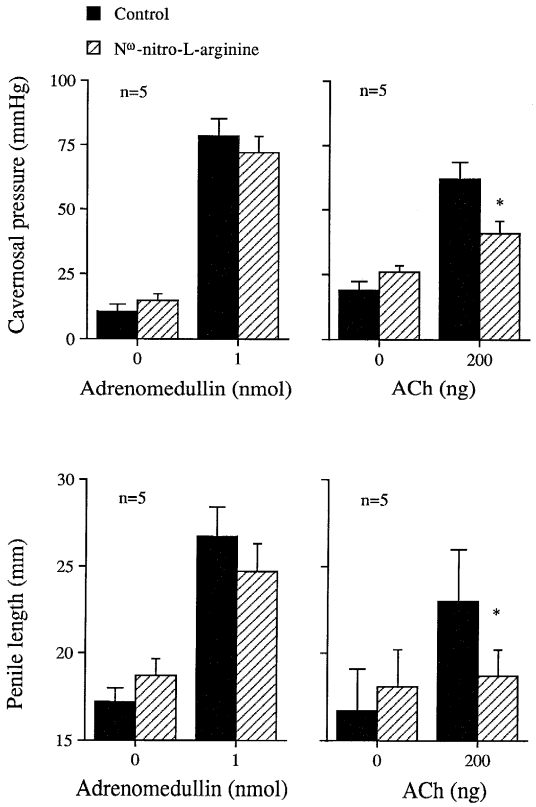

Fig. 2. Bar graphs showing the influence of the nitric oxide synthase inhibitor, N^{ω}-nitro-L-arginine, in a dose of 20 mg injected into the corpora cavernosum on erectile responses to intracavernosal injection of adrenomedullin and acetylcholine (ACh). n = number of animals. Asterisks indicate that response is significantly different from control, $P < 0.05$.

3. Results

3.1. Responses to adrenomedullin

Intracavernous injections of adrenomedullin (0.1–1 nmol) caused a dose-dependent increase in cavernosal pressure and in penile length (Fig. 1A). With the 1 nmol dose of adrenomedullin, there was approximately an 8-fold increase in cavernosal pressure (74.0 ± 10.0 mmHg) and a 43% increase in penile length (23.3 ± 1.2 mm) when compared to preinjection baseline values for cavernosal pressure (12 ± 2.5 mmHg) and penile length (16.3 ± 0.8 mm). The 1 nmol dose of adrenomedullin elicited a similar increase in penile length when compared to the response induced by the administration of the triple-drug combination. The increase in cavernosal pressure in the response to the 1 nmol dose of adrenomedullin was 74% of that obtained in response to the triple-drug combination.

Intracavernous injections of adrenomedullin (0.1–0.3 nmol) did not produce a significant decrease in systemic arterial pressure (Fig. 1C). The decreases in systemic arterial pressure induced by adrenomedullin (1 nmol) and the triple-drug combination were statistically significant ($P < 0.05$), and systemic arterial pressure returned to baseline values within 2 min after injection (Fig. 1C). The difference in decrease in systemic arterial pressure induced by the 1 nmol dose of adrenomedullin and the triple-drug combination was statistically significant (Fig. 1C). The duration of the erectile response (in minutes) was significantly shorter with the 1 nmol dose of adrenomedullin (15.6 ± 4.1) than in response to the control combination (43.0 ± 12.0; $P < 0.05$) (Fig. 1D).

3.2. Role of nitric oxide synthesis / release

The role of nitric oxide synthesis/release in mediating erectile responses to intracavernosal injection of adrenomedullin in the cat was investigated, and these data are summarized in Fig. 2. Intracavernosal injection of the nitric oxide synthase inhibitor, N^{ω}-nitro-L-arginine, in a dose of 20 mg did not significantly alter the change in cavernosal pressure and penile length in response to adrenomedullin, at a time when the erectile response to intracavernosal injection of acetylcholine was significantly reduced (Fig. 2).

4. Discussion

The feline model has been employed in investigations of the physiology and pharmacology of penile erection (Wang et al., 1993, 1994). The present study, however, is the first to demonstrate an erectile response in the feline penis in vivo in response to intracavernosal injections of adrenomedullin, a novel endogenous hypotensive peptide. Results of the present investigation show that adrenomedullin caused significant dose-related increases in both intracavernous pressure and penile length when the peptide was injected directly into the corpus cavernosum in doses from 0.1–1 nmol. The duration of action of adrenomedullin was significantly less than that observed with the triple-drug combination. In addition, the decrease in systemic arterial pressure that is commonly seen with injection of the triple-drug combination, which is used for comparative purposes, was significantly less than observed with the intracavernous injection of adrenomedullin.

Human adrenomedullin is composed of 52 amino acids with a disulfide bond that forms a six-member ring structure similar to CGRP and pancreatic amylin (Kitamura et al., 1993; Ichiki et al., 1994). Adrenomedullin decreases systemic arterial pressure in the rat and has significant vasodilator activity in the hindlimb and pulmonary vascular beds of the cat (Kitamura et al., 1993; Champion et al., 1996). The results of the present study extend previous findings by showing that adrenomedullin has significant vasodilator activity in the feline corpus cavernosum.

The mechanism by which adrenomedullin relaxes vascular smooth muscle is uncertain. Adrenomedullin, CGRP, and amylin have been reported to increase intracellular levels of cAMP (Eguchi et al., 1994a,b; Ishizaka et al., 1994). However, N^{ω}-nitro-L-arginine methyl ester (L-NAME) has been reported to inhibit responses to adrenomedullin in the hindquarters and pulmonary vascular beds of the rat and in the renal vascular bed of the dog, suggesting a role for nitric oxide in mediating the vasodilator response (Feng et al., 1994; Majid et al., 1996; Nossaman et al., 1996). Vasodilator responses to adrenomedullin in the hindlimb and pulmonary vascular beds of the cat were not attenuated by L-NAME, suggesting that the release of nitric oxide is not involved in the regional vascular bed of the cat (Champion et al., 1996; Nossaman et al., 1996). The release of nitric oxide has been shown to mediate erectile responses to acetylcholine, substance P, and other vasoactive agents (Steers, 1990; Anderson, 1993; Wang et al., 1993, 1994; Lugg et al., 1995). Inasmuch as erectile responses to adrenomedullin were not altered after administration of L-NAME at a time when responses to acetylcholine were significantly attenuated, these data suggest that nitric oxide is not involved in mediating responses to intracavernosal injection of adrenomedullin. These data are consistent with previous findings which show that adrenomedullin-induced vasodilation is independent of the synthesis/release of nitric oxide (Champion et al., 1996; Nossaman et al., 1996). Further studies, however, are needed to elucidate the mechanism of action of intracavernosal injection of adrenomedullin in the cat.

In conclusion, the results of the present study show that intracavernosal injection of adrenomedullin induces dose-related increases in cavernosal pressure and penile length. Responses to adrenomedullin were comparable to those induced by intracavernosal injection of a standard triple-drug combination composed of papaverine, phentolamine, and prostaglandin E_1. However, erectile responses to adrenomedullin were shorter in duration, and systemic hypotension was significantly less with adrenomedullin than with the triple-drug combination. Results of the present study further suggest that the erectile response induced by intracavernosal injection of adrenomedullin is not mediated by the synthesis/release of nitric oxide in the corpora cavernosum.

References

Anderson, K.E., 1993, Pharmacology of lower urinary tract smooth muscles and penile erectile tissues, Pharmacol. Rev. 45, 253.

Argiolas, A. and M.R. Melis, 1995, Oxytocin-induced penile erection, in: Oxytocin, eds. R. Ivell and J. Russell (Plenum Press, New York, NY) p. 247.

Argiolas, A., M.R. Melis and R. Stancampiano, 1993, Role of central oxytonergic pathways in the expression of penile erection, Regul. Pept. 45, 139.

Benelli, A., A. Bertolini, R. Poggioli, E. Cavazzuti, L. Calza, L. Giardino and R. Arletti, 1995, Nitric oxide is involved in male sexual behavior of rats, Eur. J. Pharmacol. 294, 505.

Bertolini, A. and G.L. Gessa, 1981, Behavioral effects of ACTH and MSH peptides, J. Endocrinol. Invest. 4, 241.

Burnett, A.L., C.J. Lowenstein, D.S. Bredt, T.S.K. Chang and S.H. Snyder, 1992, Nitric oxide: a physiologic mediator of penile erection, Science 257, 401.

Champion, H.C., J.A. Santiago, W.A. Murphy, D.H. Coy and P.J. Kadowitz, 1996, Adrenomedullin (22–52) antagonizes vasodilator responses to calcitonin gene-related peptide, but not adrenomedullin in the cat, Am. J. Physiol. (in press).

DeGroat, W.C. and A.M. Booth, 1980, Physiology of male sexual function, Ann. Intern. Med. 91, 329.

Eguchi, S., Y. Hirata, H. Assoc, K. Sago, T.X. Watanabe, T. Inui, K. Nakajima, S. Sakakibara and F. Marumo, 1994a, Structure-activity relationship of adrenomedullin, a novel vasodilatory peptide, in cultured rat vascular smooth muscle cells, Endocrinology 135, 2454.

Eguchi, S., Y. Hirata, H. Kano, K. Sago, Y. Watanabe, T.X. Watanabe, K. Nakajima, S. Sakakibara and F. Marumo, 1994b, Specific receptors for adrenomedullin in cultured rat vascular smooth muscle cells, FEBS Lett. 340, 226.

Feng, C.J., B. Kang, A.D. Kaye, P.J. Kadowitz and B.D. Nossaman, 1994, L-NAME modulates responses to adrenomedullin in the hindquarters vascular bed of the rat, Life Sci. 55, PL434.

Ichiki, Y., K. Kitamura, K. Kangawa, M. Kawamoto, H. Matsuo and T. Eto, 1994, Distribution and characterization of immunoreactive adrenomedullin in human tissue and plasma, FEBS Lett. 338, 6.

Ishizaka, Y., M. Tanaka, K. Kitamura, K. Kangawa, N. Minamino, H. Matsuo and T. Eto, 1994, Adrenomedullin stimulates cyclic AMP formation in rat vascular smooth muscle cells, Biochem. Biophys. Res. Commun. 200, 642.

Kitamura, K., K. Kangawa, M. Kawamoto, Y. Ichiki, S. Nakumura, H. Matsuo and T. Eto, 1993, Adrenomedullin: a novel hypotensive peptide isolated from human pheochromocytoma, Biochem. Biophys. Res. Commun. 192, 553.

Lugg, J.A., N.F. Gonzales-Cadavid and J. Rajfer, 1995, The role of nitric oxide in erectile function, J. Androl. 16, 2.

Majid, D.S.A., P.J. Kadowitz, D.H. Coy and L.G. Navar, 1996, Renal responses to intra-arterial administration of adrenomedullin in dogs, Am. J. Physiol. 270, F200.

Nossaman, B.D., C.-J. Feng, A.D. Kaye, B.J. DeWitt, D.H. Coy, W.A. Murphy and P.J. Kadowitz, 1996, Pulmonary vasodilator responses to adrenomedullin are reduced by nitric oxide synthetase inhibitors in the rat but not in the cat, Am. J. Physiol. 270, L782.

Steers, W.D., 1990, Neural control of penile erection, Semin. Urol. 8, 66.

Steif, C.G., F. Bernard, R. Bosch, S. Aboseif, U. Wetteraure, T.F. Lue and E.A. Tanagho, 1993, Calcitonin gene-related peptide: possibly neurotransmitter contributes to penile erection in monkeys, Urology 41, 397.

Wang, R., T.R. Higuera, S.C. Sikka, R.K. Minkes, J.A. Bellan, P.J. Kadowitz, F.R. Domer and W.J.G. Hellstrom, 1993, Penile erections induced by vasoactive intestinal peptide and sodium nitroprusside, Urol. Res. 21, 75.

Wang, R., F.R. Domer, S.C. Sikka, P.J. Kadowitz and W.J.G. Hellstrom, 1994, Nitric oxide mediates penile erection in cats, J. Urol. 151, 234.