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Lack of effect of carbon monoxide inhibitor on relaxation induced by electrical field stimulation in corpus cavernosum

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Abstract Carbon monoxide has been proposed as a possible neurotransmitter because of its ability to bind to the iron atom of the heme of guanylyl cyclase, which is similar to that of nitric oxide. To determine whether carbon monoxide exerts an effect on the penis, strips of rabbit corpus cavernosum were mounted in an organ bath for isometric tension studies and the effect of zinc deuteroporphyrin, an inhibitor of heme oxygenase which metabolizes hemoprotein and releases carbon monoxide, on relaxation induced by electrical field stimulation (neurally mediated) was determined. Also observed was relaxation induced by electrical field stimulation after incubation with atropine and guanethidine to isolate nonadrenergic noncholinergic neurotransmission. Zinc deuteroporphyrin (10^{-6} M, 10^{-5} M, 10^{-4} M and 3×10^{-4} M) did not affect relaxation induced by electrical field stimulation in the absence or presence of guanethidine and atropine. Therefore, it appears that carbon monoxide does not contribute to neurally mediated relaxation of the rabbit corpus cavernosum.

Key words Impotence · Carbon monoxide · Rabbit corpus cavernosum

Recently, nitric oxide (NO) has been identified as a major neurotransmitter modulating vascular tone and hemostasis [5]. It is known that penile erection is mediated primarily through the release of a nonadrenergic noncholinergic (NANC) neurotransmitter which has been identified as NO [1, 2]. Carbon monoxide (CO), which is generated in some tissues from hemoprotein through heme oxygenase, has similar characteristics to NO, elevating the tissue cyclic GMP content. Thus, CO has been hypothesized to serve as a neurotransmitter [7]. It has been reported that CO relaxes vascular smooth muscle and that both NO and CO serve as neurotransmitters in the hippocampus [4, 13]. If CO acts on the corpus cavernosum and if it is released by electrical field stimulation (EFS), then CO may also have a physiological role in penile erection. Therefore, we studied the effect of a CO inhibitor on the relaxation induced by EFS in corpus cavernosum to evaluate whether CO contributes to neurally mediated relaxation of corporal smooth muscle.

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Materials and methods

Male New Zealand White rabbits weighing 2–2.5 kg were used. Cavernosal tissue was procured with the rabbit under general anesthesia induced by xylazine, 30 mg/kg s.c. (Rompun, Mobay, Shawnnee, Kan., USA) and ketamine hydrochloride, 50 mg/kg s.c. (Bristol Laboratories, Syracuse, N.Y., USA). The penis was excised en block and the corpora cavernosa were dissected from the tunica albuginea and sectioned transversely, producing two strips (approximately $0.2\times0.2\times0.4$ cm) from each corpus. The rabbit was then put to death with an i.v. overdose of sodium pentobarbital (30 mg/kg).

Each cavernosal strip was placed in a 25 ml tissue bath (Kent Scientific, Litchfield, Conn., USA), tying one end to a tissue holder and the other to a force transducer (FTO3, Grass Instruments, Quincy, Mass., USA). The tissue was suspended in Krebs physiological salt solution (PSS, pH 7.4) at 37°C oxygenated with 95% O_2 and 5% CO_2. The millimolar composition of the PSS was NaCl 122, KCl 4.7, $MgCl_2$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 15.4, KH_2PO_4 1.2, glucose 5.5. Tension was monitored with a four channel polygraph (Grass 7D, Grass Instruments).

After the tissue was contracted with norepinephrine (2×10^{-5} M), EFS was performed with a 10-V square wave 0.5 ms in duration in 10-s trains at frequencies of 5, 15 and 40 Hz. Electrical field was generated using an electrical stimulator/generator (SD-9, Grass Instruments) connected to a current amplifier/splitter (Stimu-Splitter II, Med-Lab Instruments, Loveland, Colo., USA). Relaxa-

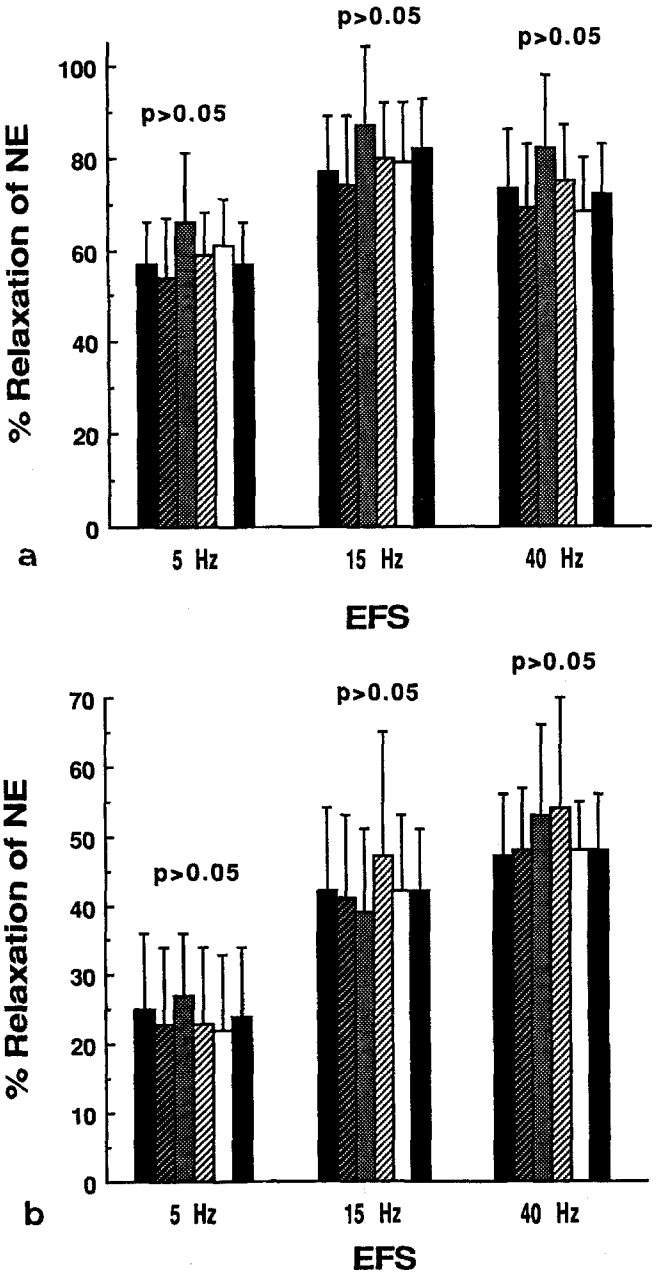

Fig. 1a, b Electrical field stimulation (*EFS*) at three frequencies (5, 15, and 40 Hz with 10-V square waves of 0.5 ms duration in 10-s trains) before and after pretreatment with an aqueous base solution for dissolving zinc deuteroporphyrin (aqueous base) and zinc deuteroporphyrin (Zn DP, 10^{-6} M, 10^{-5} M, 10^{-4} M and 3×10^{-4} M), a CO inhibitor, in the absence (**a**) and presence (**b**) of atropine and guanethidine. Heme oxygenase antagonism did not attenuate the relaxation induced by electrical field stimulation. Each point ($n=6$) represents the mean SEM percentage precontracted tension with norepinephrine (*NE*). ■ Control; ▨ aqueous base; ▩ Zn DP 10-6; ▨ Zn DP 10-5; □ Zn DP 10-4; ■ Zn DP 3×10-4

tions at three frequencies were measured in both the absence and presence of guanethidine (5×10^{-6} M, 20 min preincubation) and atropine (5×10^{-6} M, 20 min preincubation) to evaluate NANC-selective neural relaxation.

Electrical field stimulation was performed after preincubation of the aqueous base solution which was used in dissolving zinc deuteroporphyrin for 20 min to evaluate whether it altered EFS-induced relaxation in corpus cavernosum. After washing out the solution of aqueous base, EFS was repeated after preincubation with the zinc deuteroporphyrin (10^{-6} M, 10^{-5} M, 10^{-4} M and 3×10^{-4} M), an inhibitor of heme oxygenase, in both the absence and presence of guanethidine and atropine. Zinc deuteroporphyrin, a CO inhibitor, was dissolved in aqueous base. The pH of this compound in the organ bath was adjusted to 7.4. Zinc deuteroporphyrin IX was purchased from Porphyrin Products (Logan, Utah, USA) and other drugs were from Sigma (St. Louis, Mo., USA). Relaxations in response to EFS are expressed as percentages of active tension generated by norepinephrine at the optimal tension. Data are expressed as means ± SEMs with n representing the number of animals from which strips were obtained. The tissue responses to EFS, before and after preincubation with zinc deuteroporphyrin, were compared by two factor analysis of variance with repeated measures maintaining $\alpha < 0.05$ through the experiment. Results were considered to be statistically significant when $P < 0.05$.

Results

Electrical field stimulation produced frequency dependent relaxation. After pretreatment with the zinc deuteroporphyrin (10^{-6} M, 10^{-5} M, 10^{-4} M and 3×10^{-4} M), a CO inhibitor, EFS-induced relaxation was not affected ($P > 0.05$, $n=6$, Fig. 1a). Pretreatment with the zinc deuteroporphyrin did not reduce EFS-induced relaxation in the presence of atropine (5×10^{-6} M) and guanethidine (5×10^{-6} M) ($P > 0.05$, $n=5$, Fig. 1b).

Discussion

Nitric oxide has been considered a principal messenger molecule which modulates smooth muscle function and serves as a neurotransmitter in both the central and peripheral nervous system. NO elevates cyclic GMP by binding to the iron of the heme molecule at the active site of soluble guanylyl cyclase. This results in smooth muscle relaxation [5]. CO, which is generated from heme through heme oxygenase, shares some of the properties of NO. It also binds to the iron atom of hemoprotein and can exert a similar physiological role to NO, in that it can activate soluble guanylyl cyclase and elevate cyclic GMP content in the tissue [7]. CO has been shown to relax vascular smooth muscle preparation in rat heart, opossum internal anal sphincter and porcine coronary vessels [3, 8, 10]. Its effect is mediated through alteration in cellular cyclic GMP level [9] and by a decreased calcium concentration in vascular smooth muscle [4]. CO has also been shown to inhibit platelet aggregation in humans [6] and Vedernikov et al. [11] demonstrated that CO can produce similar endothelium-independent arterial relaxation to NO in the dog. Furthermore, both CO and NO have been shown to serve as retrograde messengers for long-term potentiation in the hippocampus [13].

Normal penile erection requires relaxation of the smooth muscle in the penis. This relaxation is mediated primarily by the NANC neurotransmitter, which has

recently been identified as NO [1, 2]. From the above concepts regarding the similar mechanism of action between CO and NO, it can be speculated that CO may have a physiological role in the penile erection. CO can be generated through two possible endogenous sources in the tissues; one option is catabolism of hemoprotein to biliverdin and CO through the action of heme oxygenase and the second option is peroxidative degradation of unsaturated fatty acid in the membrane [12]. The first source of CO can be regarded as a physiological effect, since heme oxygenase has been shown to be present in these organs which contain high amounts of reticulo-endothelial cells such as liver and spleen [7]. The zinc deuteroporphyrin, the heme oxygenase inhibitor, used in the current study blocks CO generation in the metabolism of hemoprotein. It had no effect on EFS induced relaxation in corpus cavernosum, suggesting that there is no conversion of heme to biliverdin with the release of CO of the rabbit corpus cavernosum. Therefore, we would imply CO does not contribute to neurally mediated relaxation in the rabbit corpus cavernosum. However, the possibility that CO may be generated from peroxidizing lipids independent of heme destruction cannot be excluded in the corpus cavernosum.

In summary, zinc deuteroporphyrin, an inhibitor of heme oxygenase, does not alter the relaxation induced by EFS in the rabbit corpus cavernosum. Thus, it does not seem likely that CO synthesis contributes to the relaxation of the corpus cavernosum.

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References

1. Brock G, Nunes L, Padma-Nathan H, Boyd S, Lue TF (1993) Nitric oxide synthase: a new diagnostic tool for neurogenic impotence. Urology 42:412
2. Burnett AL, Lowenstein CJ, Bredt DS, Chang TGK, Snyder SH (1992) Nitric oxide: a physiologic mediator of penile erection. Science 257:401
3. Gräser T, Vedernikov YE, Li DS (1990) Studies on the mechanism of carbon monoxide induced endothelium-independent relaxation in the porcine coronary artery and vein. Biomed Biochim Acta 49:293
4. Lin H, McGrath JJ (1988) Carbon monoxide effects on calcium levels in vascular smooth muscle. Life Sci 43:1813
5. Lowensein CJ, Snyder SH (1992) Nitric oxide, a novel biologic messenger. Cell 70:705
6. Mansouri A, Perry CA (1982) Alteration of platelet aggregation by cigarette smoke and carbon monoxide. Thromb Haemost 48:286
7. Marks GS, Brien JF, Nakatsu K, McLaughlin BE (1991) Does carbon monoxide have a physiological function? Trends Pharmacol Sci 12:185
8. McFaul SJ, McGrath JJ (1987) Studies on the mechanism of carbon monoxide-induced vasodilation in the isolated perfused rat heart. Toxicol Appl Pharmacol 87:464
9. Ramos KS, Lin H, McGrath JJ (1989) Modulation of cyclic guanosine monophosphate levels in cultured aortic smooth muscle cells by carbon monoxide. Biochem Pharmacol 38:1368
10. Rattan S, Chakder S (1993) Inhibitory effect of CO on internal anal sphincter: heme oxygenase inhibitor inhibits NANC relaxation. Am J Physiol 265:G799
11. Vedernikov YE, Gräser T, Vanin AF (1989) Similar endothelium-independent arterial relaxation by carbon monoxide and nitric oxide. Biomed Biochim Acta 48:601
12. Wolff DG, Bidlack WR (1976) The formation of carbon monoxide during peroxidation of microsomal lipids. Biochem Biophys Res Commun 73:850
13. Zhuo M, Small SA, Kandel ER, Hawkins RD (1993) Nitric oxide and carbon monoxide produce activity-dependent long-term synaptic enhancement in hippocampus. Science 260:1946