ELSEVIER

European Journal of Pharmacology 276 (1995) 191–194

ejp

Short communication

Cocaine promotes an apparent direct vasoconstrictor effect of neuropeptide Y in the rat tail artery

Thomas C. Glenn, Sheng-Lin Huang, Sue Piper Duckles *

Department of Pharmacology, College of Medicine, University of California, Irvine, Irvine, CA 92717, USA

Received 17 January 1995; accepted 20 January 1995

Abstract

Neuropeptide Y is a powerful vasoconstrictor in vivo; however, in vitro it shows weak constrictor effects. This discrepancy may have led to conflicting reports concerning the contractile effects of neuropeptide Y on isolated blood vessels. Using isolated rat tail and femoral artery segments neuropeptide Y (0.1–100 nM) did not induce any contractile response. However, if the catecholamine neuronal uptake blocker cocaine was added to the tissue bath, neuropeptide Y induced a contraction which could be fully blocked by prazosin (1000 nM). Furthermore, an age-dependent increase in the contraction to neuropeptide Y plus cocaine was observed. In conclusion, in the rat tail artery an apparent direct vasoconstrictor effect of neuropeptide Y occurs only in the presence of cocaine. Since this contraction can be fully blocked by prazosin, spontaneously released norepinephrine is an important component of the contraction. The discrepancy between in vivo and in vitro effects of neuropeptide Y may be explained in part by the presence of circulating vasoconstrictors.

Keywords: Aging; Noradrenaline release, spontaneous; Vasoconstrictor; Potentiation

1. Introduction

Neuropeptide Y is a 36 amino acid peptide co-transmitter with norepinephrine in peripheral adrenergic nerves (Lundberg et al., 1983). Neuropeptide Y has been shown to be a powerful vasoconstrictor in vivo but appears to be less effective in vitro (for review see Zukowska-Grojec and Wahlestedt, 1993). Neuropeptide Y has been reported to act both as a direct vasoconstrictor and as a nonselective potentiator of various vasoconstrictor agents which include norepinephrine, endothelin, and potassium (Edvinsson et al., 1984; MacLean and Hiley, 1990; Andriantsitohaina and Stoclet, 1988). Numerous factors appear to influence the ability of neuropeptide Y to cause vasoconstriction, including anatomical size and location of blood vessels. For example, feline cerebral arteries have been shown to contract to neuropeptide Y in the presence of adrenergic receptor blockade (Edvinsson, 1985). Furthermore, in small vessels such as the main side branch of the central rabbit ear artery, neuropeptide Y is more likely to act as a direct vasoconstrictor than in larger blood vessels (Owen, 1993).

There are reported discrepancies, however, as to whether or not neuropeptide Y acts as a direct vasoconstrictor. For example, Neild (1987) has reported that neuropeptide Y (30–1000 nM) can cause a slowly developing vasoconstriction in the rat tail artery whereas Vu et al. (1989) were unable to observe such a response with neuropeptide Y (10–100 nM) and observed only a potentiation of the contractile responses to adrenergic nerve stimulation or addition of exogenous norepinephrine. We and others have recently shown that neuropeptide Y preferentially potentiates contractile responses to lower concentrations of norepinephrine as well as lower frequencies of adrenergic nerve stimulation (Fallgren et al., 1993; Glenn and Duckles, 1994). Given this ability of neuropeptide Y to potentiate other vasoconstrictors particularly when they exist at low concentrations, we hypothesize that subpressor levels of vasoconstrictors such as norepinephrine must be present for the apparent direct vasoconstrictor effects of neuropeptide Y. To test our hy-

* Corresponding author. Department of Pharmacology, College of Medicine, University of California, Irvine, Irvine, CA 92717, USA. Tel. 714-856-4265, fax 714-856-4855.

SSDI 0014-2999(95)00050-X

pothesis we used the adrenergic uptake blocker cocaine to elevate basal levels of norepinephrine in isolated rat tail artery segments which were then tested for neuropeptide Y responses. Additionally, by using the α-adrenoceptor antagonist prazosin we further studied the contribution of norepinephrine to the neuropeptide Y response. Finally, we performed the studies in tail arteries from three ages of Fischer 344 rats to determine if age alters the response to neuropeptide Y.

2. Materials and methods

2.1. Tissue isolation and preparation

Male F344 rats ages 4 (289 ± 8 g), 12 (417 ± 10 g), and 24 months (386 ± 9 g) (NIH-NIA colony operated by Harlan Sprague Dawley, Indianapolis, IN, USA) were decapitated and proximal tail arteries removed and cleaned of excess connective tissue. Arteries were cut into 3 mm ring segments, and platinum wires were carefully inserted through the lumen of the rings.

2.2. Measurement of isometric contraction

Isometric force measurements were made from tail artery ring segments mounted in 50 ml tissue baths containing oxygenated Krebs buffer (NaCl 118 mM; KCl 4.8 mM; $CaCl_2$ 1.6 mM; KH_2PO_4 1.2 mM; $NaHCO_3$ 25 mM; $MgSO_4$ 1.2 mM; ascorbic acid 0.3 mM; and glucose 11.5 mM) at 37°C. An optimal resting tension for the age groups studied here was applied to all segments (Duckles, 1987). Isometric force was measured by a Gould Statham UC2 transducing cell connected to a Maclab analog digital convertor and Macintosh computer.

2.3. Drugs and chemicals

All drugs were added directly into the tissue baths containing the artery rings. Neuropeptide Y was purchased from Peninsula Labs, Belmont, CA, USA, and cocaine hydrochloride and prazosin hydrochloride were purchased from Sigma Chemicals, St. Louis, MO, USA.

3. Results

3.1. Actions of neuropeptide Y and cocaine

Neuropeptide Y in concentrations from 0.1 to 100 nM did not induce a contractile response in rat tail artery segments regardless of age (Fig. 1A, $n = 15$). Cocaine (10^{-5} M) also had no contractile effect alone; however, if cocaine was added to the tissue bath after a concentration response with norepinephrine had been established, even after repeated washing, a contraction of 0.2 g upon addition of cocaine was observed ($n = 2$). In a norepinephrine naive bath containing cocaine the addition of neuropeptide Y (2×10^{-8} M) now produced a contraction (Fig. 1B and Table 1; $n = 15$). A similar response was seen in isolated femoral arteries where the combination of cocaine and neuropeptide Y (10^{-8} M) produced a contraction of 0.25 ± 0.12 g ($n = 2$). This contractile response to neuropeptide Y in the presence of cocaine was observed regardless of the order of addition of the two compounds to the tissue bath. The combined effect of neuropeptide Y plus

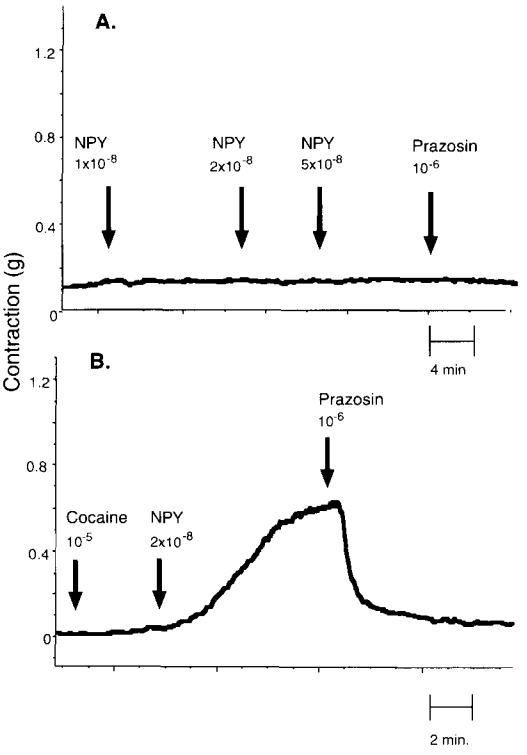

Fig. 1. Neuropeptide Y (NPY) produced contraction in the presence of cocaine which was blocked by prazosin. (A) Representative tracings show the lack of contractile response of tail artery segments to neuropeptide Y ($1\text{–}5 \times 10^{-8}$ M), cocaine (10^{-5} M), or prazosin (10^{-6} M) alone. (B) In contrast in the presence of cocaine, neuropeptide Y (2×10^{-8} M) produced a contractile response of 0.6 g. Prazosin (10^{-6} M) completely inhibited the response.

Table 1
Contractile response to neuropeptide Y (2×10^{-8} M) plus cocaine (10^{-5} M) in the rat tail artery

Age (months)	Contractile response (g)
4	0.08 ± 0.02
12	0.37 ± 0.09 [a]
24	0.52 ± 0.11 [a]

Contractions are expressed as grams of isometric tension. [a] Different from one age group, $P < 0.05$, ANOVA, $n = 5$.

cocaine was consistently seen in all tail and femoral artery segments studied ($n = 17$).

3.2. Antagonism by prazosin

Prazosin (10^{-6} M) by itself did not affect the resting tension of the rat tail artery (Fig. 1A, $n = 5$). However, prazosin completely inhibited the contractile response to cocaine plus neuropeptide Y ($n = 5$).

3.3. Influence of age

The magnitude of the contractile response to neuropeptide Y in the presence of cocaine was age dependent. Contraction caused by neuropeptide Y together with cocaine was significantly greater in 12- and 24- than in 4-month-old F344 rat tail artery rings (Table 1, $n = 5$). This cannot be explained merely as a difference in contractile responsiveness as ring segments from all three ages responded similarly to a maximal concentration of norepinephrine (7×10^{-5} M) (4 months, 1.94 ± 0.4 g; 12 months, 2.2 ± 0.2 g; 24 months 1.8 ± 0.4 g; $n = 5$). Neither neuropeptide Y alone nor cocaine alone caused any change in the isometric tension regardless of age ($n = 5$ in each age group).

4. Discussion

In this study, varying concentrations of neuropeptide Y (0.1–100 nM) alone did not induce a contractile response. However, in the presence of cocaine, which blocks the re-uptake of norepinephrine, neuropeptide Y caused a marked contractile response. Furthermore, addition of the α_1-adrenoceptor antagonist prazosin completely inhibited the response to cocaine plus neuropeptide Y, indicating that norepinephrine is present and involved in the contractile response. Thus blockade by prazosin of the response to neuropeptide Y in the presence of cocaine suggests that when norepinephrine uptake is blocked, spontaneously released norepinephrine reaches significant levels in this isolated tissue. Therefore this study shows that under these conditions an apparent direct vasoconstrictor action of neuropeptide Y requires the presence of another agonist, albeit at a subthreshold level.

The ability of neuropeptide Y to non-selectively potentiate vasoconstrictor agents has been well documented. We have recently shown in the rat tail artery that an inverse relationship exists between the magnitude of the contractile response to nerve stimulation and the percent potentiation caused by neuropeptide Y (Glenn and Duckles, 1994). Therefore, at extremely low concentrations of norepinephrine, neuropeptide Y would be expected to induce the greatest level of potentiation. It has been suggested that a 'threshold synergism phenomenon' may be responsible for the cooperativity or potentiation seen with neuropeptide Y and norepinephrine (Wahlestedt et al., 1990). However, in the tail artery we do not observe any direct contractile response to neuropeptide Y alone, and therefore it is unlikely that a 'threshold' level of neuropeptide Y is present. Therefore, the most plausible role for neuropeptide Y in this tissue is solely as a potentiator.

This study underscores that discrepancies seen between different reports may be due to different experimental conditions. For example, pre-treatment of tissue segments with norepinephrine to establish an initial concentration response curve may create a reservoir of norepinephrine which may serve as a source of subthreshold levels of vasoconstrictor. In support of this we found that addition of cocaine following norepinephrine exposure and thorough washout still produced a small contraction.

The observation that the response to neuropeptide Y plus cocaine was greater in older animals suggests several possible explanations. One possibility is that the presynaptic uptake of norepinephrine is diminished with age thereby increasing the concentration of norepinephrine in the synapse. This is unlikely as it has previously been shown that reuptake and metabolism of norepinephrine do not change with age (Duckles, 1987; Buchholz and Duckles, 1990). A second possibility is that the younger vessels cannot achieve the same level of contractile force as the older animals. However, we have shown in the present study and in previous studies that the maximal contraction of tail artery does not change with age thereby eliminating the possibility of age-related differences in the ability of the blood vessels to contract (Duckles et al., 1985). Furthermore it has been shown that the sensitivity to vasoconstrictors such as norepinephrine does not vary with age in the rat (Duckles et al., 1985). Another possibility is that an age-related increase in the sensitivity to neuropeptide Y occurs. We have recently shown that sensitivity to the potentiating effects of neuropeptide Y at concentrations up to 10^{-8} M does indeed increase with age, reaching a plateau at 12 months which is then maintained through 24 months (Glenn and Duckles, 1994). Therefore, it is likely that the age-related differences in response to neuropeptide Y plus cocaine are due, at least in part, to the greater potentiation caused by neuropeptide Y in older animals. A fourth possibility is that the level of spontaneously released norepinephrine may be greater in older animals. Indeed, we have demonstrated that stimulation-evoked norepinephrine release increases with age in the rat tail artery; however, we have no information on the effect of age on basal norepinephrine release as this is below the sensitivity of our detection system (Buchholz and Duckles, 1990). Thus

we cannot rule out the possibility that there is also a greater spontaneous release of norepinephrine.

In conclusion, the apparent direct vasoconstrictor effects of neuropeptide Y in the rat tail artery appear to require the presence of subthreshold levels of a vasoconstrictor such as norepinephrine. Since neuropeptide Y can non-selectively potentiate a wide range of vasoconstrictor agents it is possible that the powerful in vivo actions of neuropeptide Y are due to the presence of low levels of circulating vasoconstrictors. Effects of prazosin, which completely abolished the contractile response to cocaine plus neuropeptide Y in our experiments, as well as other antagonists of endogenous vasoconstrictor substances may help to clarify the role of neuropeptide Y in vasoconstriction both in vivo and in vitro.

Acknowledgments

The authors wish to gratefully acknowledge the assistance of Dr. Diana Krause in reviewing the manuscript. This work was supported in part by NIH grant No. R01H250775.

References

Andriantsitohaina, R. and R. Stoclet, 1988, Potentiation by neuropeptide Y of vasoconstriction in rat resistance arteries, Br. J. Pharmacol. 95, 419.

Buchholz, J. and S.P. Duckles, 1990, Effect of age on prejunctional modulation of norepinephrine release, J. Pharmacol. Exp. Ther. 252, 159.

Duckles, S.P., 1987, Effect of norepinephrine uptake blockade on contractile responses to adrenergic nerve stimulation of isolated rat blood vessels: influence of age, J. Pharmacol. Exp. Ther. 243, 521.

Duckles, S.P., B.J. Carter and C.L. Williams, 1985, Vascular adrenergic neuroeffector function does not decline in rats, Circ. Res. 56, 109.

Edvinsson, L., 1985, Characterization of the contractile effect of neuropeptide Y in feline cerebral arteries, Acta Physiol. Scand. 125, 33.

Edvinsson, L., E. Ekblad, R. Håkanson and C. Wahlestedt, 1984, Neuropeptide Y potentiates the effect of various vasoconstrictor agents on rabbit blood vessels, Br. J. Pharmacol. 83, 519.

Fallgren, B., P. Arlock and L. Edvinsson, 1993, Neuropeptide Y potentiates noradrenaline-evoked vasoconstriction by an intracellular calcium-dependent mechanism, J. Auton. Nerv. Syst. 44, 151.

Glenn, T.C. and S.P. Duckles, 1994, Vascular responses to neuropeptide Y in the rat: effect of age, Aging Clin. Exp. Res. 6, 277.

Lundberg, J.M., L. Terenius, T. Hökfelt and M. Goldstein, 1983, High levels of neuropeptide Y in peripheral noradrenergic neurons in various mammals including man, Neurosci. Lett. 42, 167.

Maclean, M.R. and C.R. Hiley, 1990, Effect of neuropeptide Y on cardiac output, its distribution, regional blood flow and organ vascular resistances in the pithed rat, Br. J. Pharmacol. 99, 340.

Neild, T.O., 1987, Actions of neuropeptide Y on innervated and denervated rat tail arteries, J. Physiol. 386, 19.

Owen, M.P., 1993, Similarities and differences in the postjunctional role for neuropeptide Y in sympathetic vasomotor control of large vs. small arteries of rabbit renal and ear vasculature, J. Pharmacol. Exp. Ther. 265, 887.

Vu, H.Q., D. Budai and S.P. Duckles, 1989, Neuropeptide Y preferentially potentiates responses to adrenergic nerve stimulation by increasing rate of contraction, J. Pharmacol. Exp. Ther. 251, 852.

Wahlestedt, C., R. Håkanson, C.A. Vaz and Z. Zukowska-Grojec, 1990, Norepinephrine and neuropeptide Y: vasoconstrictor cooperation in vivo and in vitro, Am. J. Physiol. 258, R736.

Zukowska-Grojec, Z. and C. Wahlestedt, 1993, Origins and actions of neuropeptide Y in the cardiovascular system, in: The Biology of Neuropeptide Y and Related Peptides, eds. W.F. Colmers and C. Wahlestedt (Humana Press, Totowa) p. 315.