Adrenergic Mechanisms in Cerebral Blood Vessels: Effect of Tyramine on the Isolated Middle Cerebral Artery of the Goat

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Abstract: *Adrenergic Mechanisms in Cerebral Blood Vessels: Effect of Tyramine on the Isolated Middle Cerebral Artery of the Goat*

■ Tyramine induces dose-dependent changes in tension of the isolated middle cerebral artery of the goat. Cocaine, phentolamine and reserpine reduce the sensitivity of the tissue to tyramine by factors of 2.8, 3.7 and 3.7, respectively. The norepinephrine (NE) concentration of the arteries of the circle of Willis is 2.10 μg per gram and the corresponding value for the right atria is 1.25 μ per gram. Reserpine pretreatment (0.02 mg/kg/day for three days) reduces the NE concentration of the cerebral arteries to undetectable levels and that of the right atria to 2.4% of the control value. The relatively high concentration of NE of the cerebral arteries of the goat suggests that this tissue receives considerable sympathetic innervation. It is likely that part of the contractile response to tyramine is due to release of endogenous NE from sympathetic stores in the artery. However, some contractile response to tyramine remains after α-adrenergic blockade, reserpine pretreatment and in the presence of cocaine, suggesting that in addition to an indirect action (release of NE) tyramine also possesses a direct stimulatory effect in cerebral arteries.

Additional Key Words
cocaine
sympathetic
phentolamine
innervation
reserpine

□ It has recently been shown in the unanesthetized goat that tyramine administered directly into the arterial supply of one brain hemisphere, or electrical stimulation of the cervical sympathetic chain, produces reductions in cerebral blood flow which are partially prevented by reserpine pretreatment or by alpha adrenergic blockade.[1, 2] These results have been interpreted in terms of release, by tyramine or electrical stimulation, of endogenous norepinephrine most likely associated with the sympathetic innervation of the cerebral blood vessels. The presence of sympathetic nerve terminals in the cerebral vasculature of other animal species has been reported in several morphological studies.[3-7]

In order to test, experimentally, the above-mentioned interpretation the present work was undertaken to assess whether: (1) norepinephrine is present in the walls of the cerebral blood vessels of the goat, (2) tyramine induces contraction in these vessels under in vitro conditions, and (3) endogenous norepinephrine contributes to the mechanism of action of tyramine on this vascular tissue.

Methods

Goat brains were obtained from the local slaughter house within one hour of the death of the animal. The middle cerebral artery was carefully dissected out and cut into cylindrical segments of 5-mm length. The cylinder was set up for isometric recording in an organ bath according to the method described by Nielsen and Owman.[8] Briefly, the method consists of passing two fine stainless steel pins through the lumen of the vascular segment. One pin is fixed to the organ bath wall while the other is connected to strain gauge for isometric recording. The latter pin is in a parallel position with the former, and is movable, thus permitting the application of resting tension in a perpendicular plane to the long axis of the vascular cylinder. The recording system included a Universal Transducing Cell UC3, a Statham Micro-Scale Accessory U15, and a Beckman Type RS recorder.

The organ bath contained 3 ml of Krebs-Henseleit solution continuously bubbled with 95% oxygen and 5% carbon dioxide, pH 7.3 to 7.4, and kept at 37°C. The composition of the Krebs-Henseleit solution was (mM): NaCl, 115; KCl, 4.6; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $MgSO_4$, $7H_2O$, 1.2; $NaHCO_3$, 2.5; glucose, 11.1. A resting tension of 1 gm was applied to the tissue and readjusted every 15 minutes during a one-hour period of equilibration. Dose-response curves to tyramine were determined in a cumulative manner, and control and experimental responses were obtained from separate vascular preparations. Tyramine was dissolved in saline

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solution containing ascorbic acid 0.01% (w/v); the total volume added to the bath did not exceed 0.3 ml. When cocaine or phentolamine was used, these agents were added to the bath 10 minutes before tyramine and allowed to remain in contact with the tissue throughout the determination of the dose-response relationship.

Reserpine was administered i.v. in a dose of 0.02 mg/kg/day for three days. Twenty-four hours after the last dose of reserpine the animals were killed by injecting i.v. 5 ml of saturated solution of potassium chloride. The right atria and the arteries of the circle of Willis, both from control and reserpine-treated goats, were taken for the fluorometric determination of the norepinephrine tissue concentration according to the method of Shellenberger and Gordon.[9] It was necessary to pool the cerebral arteries of three goats in order to have enough tissue for the fluorometric analysis. Tissue weight per pool ranged from 100 to 125 mg. Recovery of norepinephrine was 60% and the tissue values were corrected accordingly.

Some segments were taken from the vascular samples to study the contractile effects of tyramine and the remaining samples were pooled to determine norepinephrine tissue levels.

Geometric means of equieffective doses were determined in the manner described by Fleming et al.[10] The 400-mg level (effective dose 400 mg) was selected for quantitative analysis because it corresponded approximately to half the maximum tension achieved with the highest concentration of tyramine used. Horizontal displacement of the dose-response curves was measured at this level by determining the ratio of ED 400 mg experimental over ED 400 mg control.

Statistical analysis was done by means of Student's *t* test, considering as significant a probability value of less than 5%. Drugs used were: tyramine hydrochloride (Sigma), phentolamine methanesulfonate (Ciba), reserpine (Ciba), and cocaine hydrochloride (Lab. Abello).

Results

Figure 1 illustrates the effect of cocaine 10^{-4}M upon the response of the isolated middle cerebral artery to tyramine. The changes in tension induced by the first three doses of tyramine were significantly reduced by cocaine ($P < 0.05$, < 0.05, < 0.02, respectively), but the response to the highest dose of tyramine used in these experiments was not significantly modified

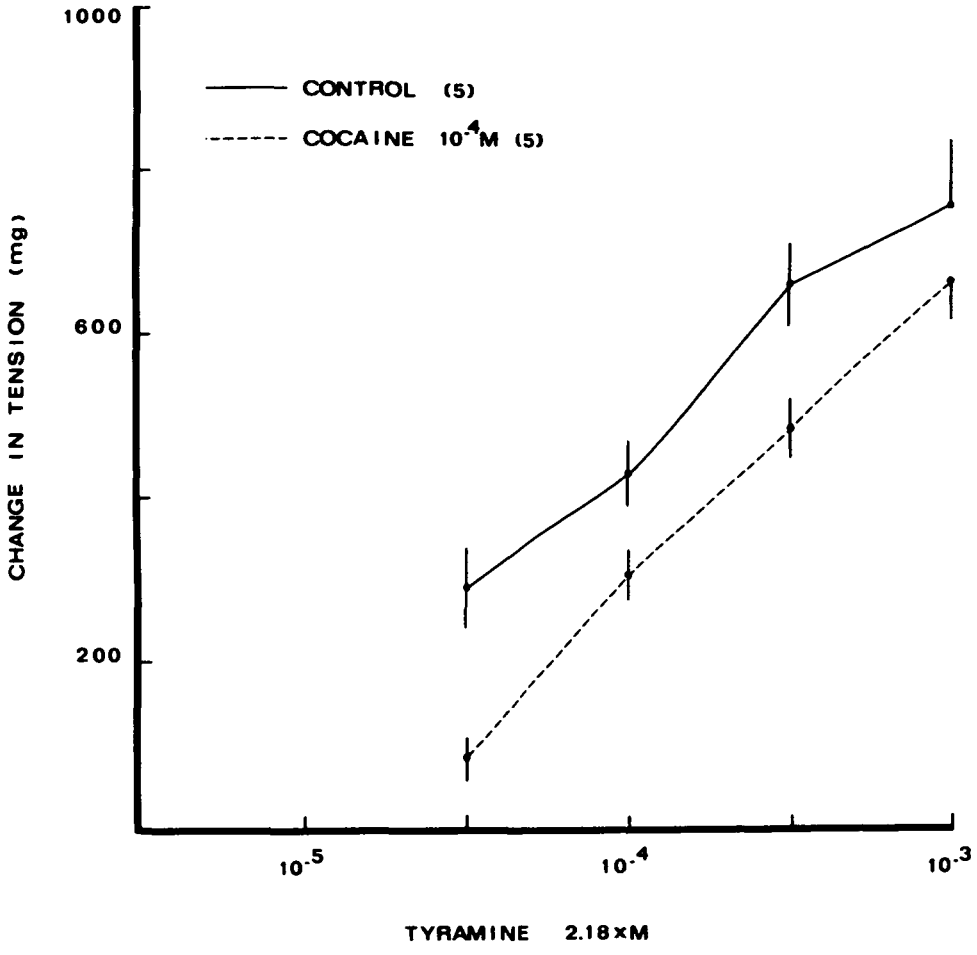

FIGURE 1

Dose-response curves for tyramine determined in the absence and in the presence of cocaine 10^{-4}M. Figures in parentheses refer to number of experiments. Vertical bars represent standard errors of the means.

TABLE 1

Effects of Cocaine, Phentolamine and Reserpine on the ED 400 mg and Maximum Response to Tyramine in the Isolated Middle Cerebral Artery of the Goat

Group	Geometric mean ED 400 mg (95% confidence limits) $\times 10^{-4}$M	ED 400 mg experimental / ED 400 mg control	Maximum response* mg ± SE	No. experiments
Control	1.50 (0.87 – 2.58)		758 ± 78	5
Cocaine	4.26 (2.75 – 6.60)†	2.8	664 ± 45	5
Phentolamine	5.63 (2.14 – 14.77)†	3.7	485 ± 58†	8
Reserpine	5.68 (3.67 – 8.82)†	3.7	660 ± 62	5

*Refers to response obtained with tyramine 2.18 $\times$ 10^{-3}M.
†Different from control, $P < 0.05$.

($P < 0.6$). The control dose-response curve for tyramine was shifted 2.8-fold to the right by cocaine (table 1).

The effect of phentolamine 10^{-6}M on the tyramine-induced changes in tension are shown in figure 2. Phentolamine displaced the dose-response curve for tyramine 3.7-fold to the right of control, with a tendency for parallelness but with a slight decrease in slope at the latter part of the curve (see also table 1). Note that phentolamine reduced significantly the responses to all doses of tyramine ($P < 0.025$, < 0.05, < 0.02, < 0.02, from low to high

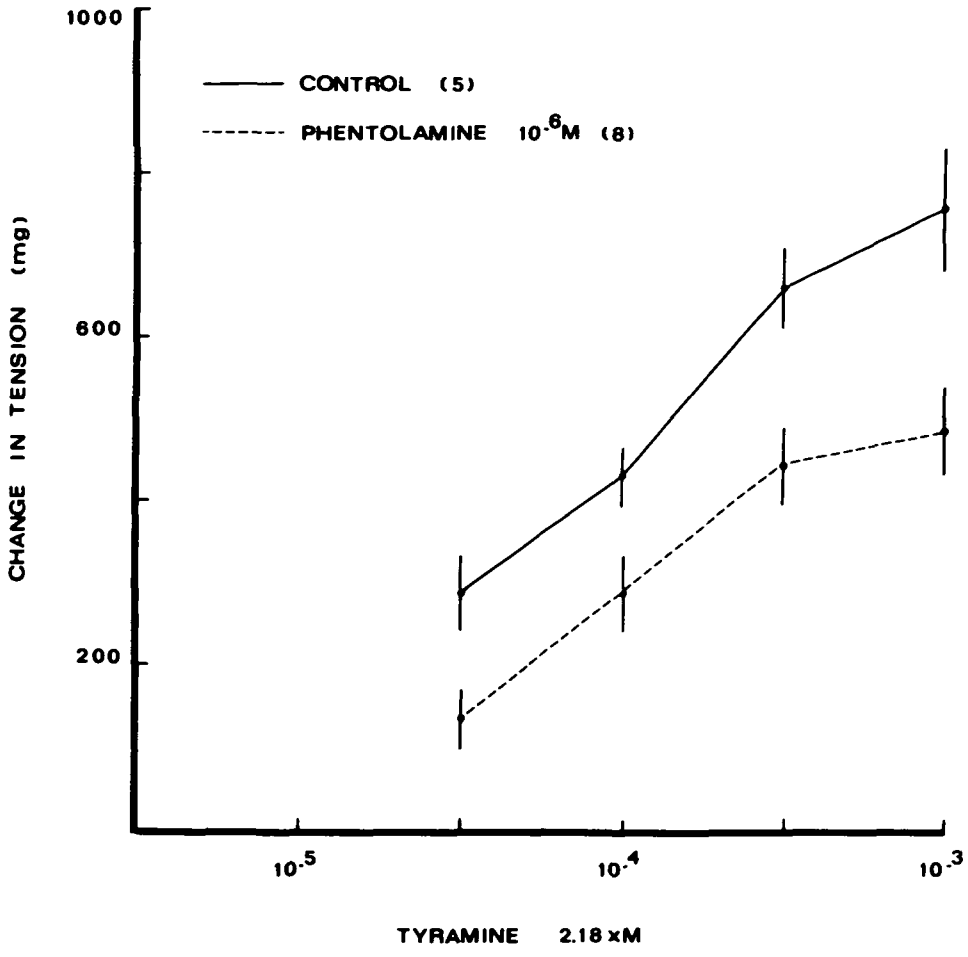

FIGURE 2

Dose-response curves for tyramine determined in the absence and in the presence of phentolamine 10^{-6}M. Number of experiments are indicated in parentheses. Vertical bars represent standard errors of the means.

doses, respectively). Although the maximum response to tyramine has been reduced by phentolamine, comparison of equieffective doses has been done at the 400-mg level of response because this tension change lies in the parallel portion of the curves, and for the sake of uniformity since the effects of the other treatments have been measured at this level.

Figure 3 shows the dose-response curves for tyramine determined in tissues from control and reserpine pretreated animals. The responses to the first three doses of tyramine were reduced by reserpine pretreatment ($P < 0.02$, < 0.005, < 0.05, respectively), whereas that due to the highest dose was not altered ($P < 0.3$). At the ED 400-mg level, reserpine pretreatment induced a 3.7-fold shift to the right of the control curve for tyramine (table 1).

In order to estimate the catecholamine-depleting ability of reserpine in the cardiovascular system of the goat, norepinephrine tissue levels were measured in the right atria and cerebral arteries of control and reserpine pretreated animals. In control animals, the right atria contained 1.25 μg of norepinephrine per gram of tissue, whereas the corresponding value for the cerebral vessels was 2.10 μg per gram. Reserpine reduced the norepinephrine concentrations of the cerebral arteries and of the right atria to undetectable levels and to 2.4% of control, respectively (table 2).

Discussion

The present study shows that the cerebral arteries of the goat possess a relatively high concentration of norepinephrine (NE). The NE concentration found in the goat cerebral vessels was greater than the corresponding values for the goat right atria (table 2), the rabbit aortic strips,[11] or the rabbit ear artery.[12] Since tissue levels of NE appear to be associated with the density of adrenergic innervation,[13-15] it is likely that the high concentration of NE of the goat cerebral

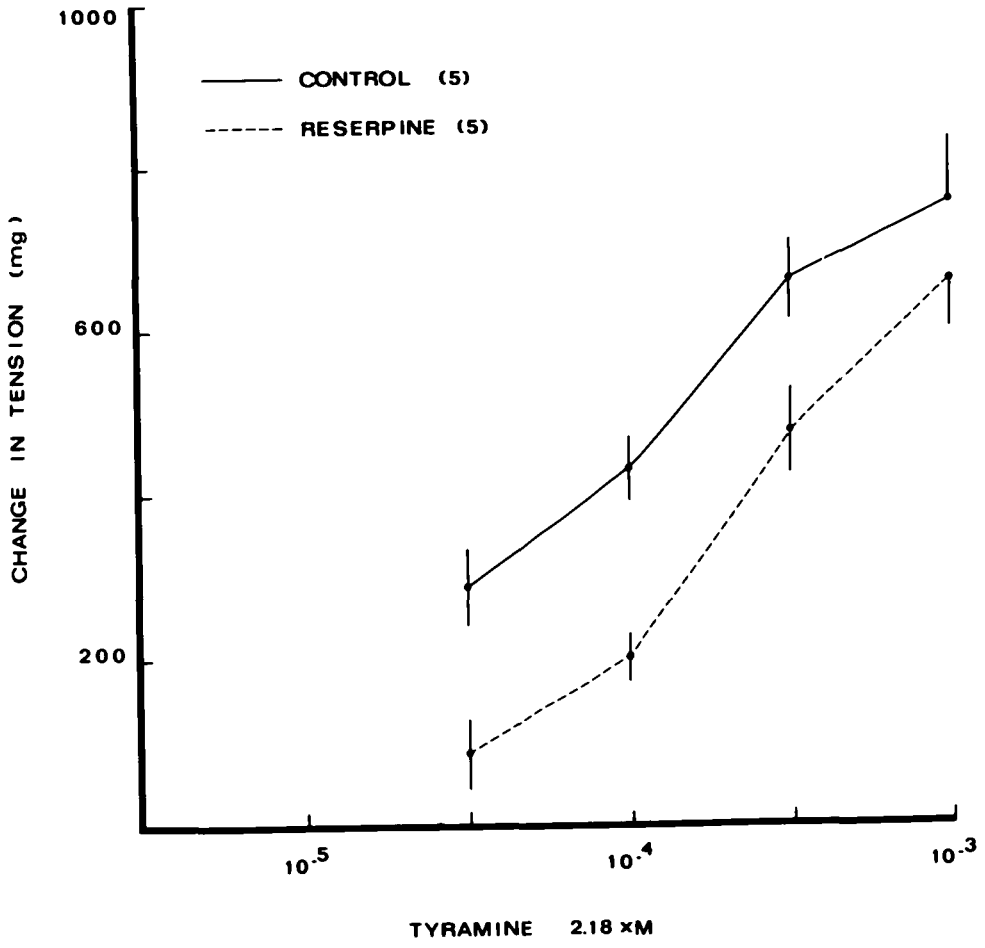

FIGURE 3

Dose-response curves for tyramine determined in tissues from control and from reserpine-pretreated animals. Reserpine pretreatment: 0.02 mg/kg/day i.v. for three days. Number of experiments are indicated in parentheses. Vertical bars represent standard errors of the means.

TABLE 2

Effect of Reserpine Pretreatment on the Norepinephrine (NE) Tissue Concentration of the Right Atria and Cerebral Arteries of the Goat

Group	NE tissue content μg per gram ± SE	
	Right atria	Cerebral arteries
Control	1.25 ± 0.09 n = 7*	2.10 ± 0.08 n = 3 pools
Reserpine (0.02 mg/kg/day, three days)	0.03 ± 0.007 n = 5	Undetectable† n = 3 pools

*"n" refers to number of animals in the case of right atria and to number of pools (three animals per pool) in the case of cerebral arteries.

†$\frac{\text{Fluorescence of tissue sample}}{\text{Fluorescence of tissue blanks}} < 1.0$.

arteries reflects the rich sympathetic supply received by this vascular tissue. The finding that a low dose of reserpine administered for a short period of time produced a marked catecholamine-depleting effect in both the atria and cerebral vessels suggests that the turnover rates of the endogenous stores of NE in these tissues are relatively high.[16, 17]

The response of the cerebral arteries to tyramine is apparently mediated to some extent by the release of adrenergic transmitter. The evidence for this is that reserpine pretreatment, which reduced endogenous NE to undetectable levels, reduced the response to tyramine. However, in spite of the absence of NE in the vascular tissues obtained from animals pretreated with reserpine, tyramine still produced a contractile effect. The dose-response curve for tyramine was only 3.7-fold to the right of the control. These results raise the possibility that the effects of tyramine on the cerebral vasculature of the goat include, in addition to its well-known indirect mechanism of action mediated through the release of NE,[18] another component which appears to be independent of the endogenous stores of adrenergic transmitter. This interpretation is supported by the results obtained with cocaine, an agent generally considered to interfere with the neuronal uptake of amines[19, 20] and which, therefore, would prevent the access of tyramine to the storage sites of endogenous norepinephrine. In the present study, cocaine was used in a concentration ten times higher than that shown to block the uptake of labeled norepinephrine in rabbit aortic strips[21] and it produced a 2.8-fold shift to the right of the tyramine control curve. The reduction of the response to tyramine induced by cocaine was comparable to the reduction produced by reserpine, and the ED 400 mg of tyramine under both procedures was not significantly different ($P < 0.1$). Based on this evidence, tyramine seems to possess a mechanism of action which is independent of the availability of adrenergic neurotransmitter. These results differ from those obtained by Nielsen et al.[22] on the isolated middle cerebral artery of the cat. Those authors showed that reserpine pretreatment or removal of the superior cervical ganglion virtually eliminated the responses to tyramine. However, it is interesting to note in their study that two weeks after denervation the response to tyramine returned to control levels. Nielsen et al.[22] explain this finding in terms of the development of supersensitivity to the remaining direct effect of tyramine, thus implying a mixed mechanism of action of tyramine in the cat cerebral vessels.

Phentolamine 10^{-6}M, a concentration which reduces the sensitivity of rabbit aortic strips to norepinephrine to one-tenth of normal,[23] produced approximately the same degree of displacement of the tyramine control curve as does reserpine or cocaine (table 1). These data indicate that norepinephrine released by tyramine induces contractile effects through the activation of alpha adrenergic receptors. The present results also suggest that the potency of phentolamine is lower in the goat cerebral arteries than in the rabbit aortic strips. This may be due to differences in the density of adrenergic innervation in the two arteries as inferred from their respective norepinephrine concentration (present study table 2; ref. 11). The density and spatial distribution of the nerve endings in rabbit aortic strips seem to influence the potency of phentolamine against endogenously released norepinephrine.[21] A further indication of this study is that the direct component of the effect of tyramine does not appear to involve alpha adrenergic receptors because if this were the case one would expect that phentolamine exerted a greater blockade compounded by antagonism to the action of the released mediator (NE) and to the action of the releasing agent (tyramine). Possibly, another type of receptor system mediates the direct effect of tyramine in this preparation, as it has been suggested for other tissues.[24] At high doses of tyramine, however, some degree of activation of alpha adrenergic receptors begins to appear since phentolamine significantly reduced the response to 2.18×10^{-3}M (fig. 2 and table 1). Thus, the effects of tyramine on the isolated middle cerebral artery of the goat involve, conceivably, the release of endogenous norepinephrine present in the artery wall, plus another mechanism of action which is likely to be nonadrenergic in nature.

In summary, the presence of the adrenergic neurotransmitter in the cerebral arteries of the goat, the catecholamine-depleting ability of reserpine and the indirect component of action of tyramine in these blood vessels are experimental facts which lend support to the proposal of Lluch et al.[1, 2] pointing toward the possible role of the sympathetic nervous system in the regulation of cerebral blood flow of the goat. D'Alecy and Feigl[25] have made a similar proposal for the sympathetic nervous control of the cerebral blood flow of the dog.

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