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Vasoconstriction of the Isolated Communicating Cerebral Artery Induced by Field Electrical Stimulation *

J. Marín and M. Salaices

Departamento de Farmacología y Terapéutica
Facultad de Medicina
Universidad Autónoma
Madrid - 34

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The vasoconstrictor effect elicited by field electrical stimulation of the posterior communicating cerebral artery of the goat was analyzed before and after treatment with pharmacological agents to find out if the adrenergic system was involved in this response. For this purpose, trains of 300 square wave pulses (1-32 Hz, 0.5 msec.) at supramaximal voltage were applied to these arteries producing a frequency-dependent increase in tension. The vasoconstrictor response was significantly reduced by tetrodotoxin (3×10^{-6} M), phentolamine (10^{-6} M) and bretylium (5×10^{-4} M), but it was not modified by cocaine (10^{-6} M). The contraction produced by electrical stimulation of arterial segments from goats pretreated with reserpine (0.02 mg/kg/day for three days) and from goats on which a bilateral superior cervical gangliectomy had been performed 12 days previously, was significantly reduced as compared with controls. These results show that a large part of the vasoconstrictor response of the goat cerebral vessels to field electrical stimulation is mediated by an adrenergic mechanism.

Various methods have been used to demonstrate the presence of adrenergic fibers containing catecholamines in the wall of intracranial arteries of various animal species, including man (2, 9, 10, 12, 17, 19, 21). These fibers have their origin mainly in the superior cervical sympathetic ganglia, since gangliectomy induces a reduction of the perivascular nerve fiber density (6, 17), an increase in cerebral blood flow (1, 10, 11), a decrease in the contractile response of the isolated cerebral artery to tyramine (20) and to electrical stimulation (5, 14). Furthermore, pre-

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treatment with reserpine caused the specific fluorescence and the amount of noradrenaline of pial arteries to disappear (7, 9, 23). *In vivo* studies have shown that intravascular administration of tyramine or electrical stimulation of the cervical sympathetic nerves produce a decrease in cerebral blood flow. This effect diminishes with the administration of reserpine or phentolamine (1, 15). In addition, when the superior cervical sympathetic ganglia were removed, an enhancement of cerebral blood flow resulted (1).

These extensive studies suggest that the brain vessels possess a sympathetic tone. Nevertheless, when the electrical stimulation of isolated cerebral arteries has been used to demonstrate the existence of this tone in cerebral arteries, the results obtained have been conflicting. Thus while some investigators have reported vasoconstrictor effects to electrical stimulation in brain arteries of different animal species mediated by an adrenergic mechanism (5, 8, 14, 16), others have reported no effects at all (4, 22). The aim of the present study therefore was to analyze the adrenergic mechanisms involved in the contractile response induced by field electrical stimulation of the communicating cerebral artery of the goat.

Materials and Methods

Female goats, ranging in weight from 30 to 45 kg, were killed by injecting i.v. 15 ml of saturated solution of KCl. Their brains were carefully removed and their posterior communicating cerebral artery dissected into cylindrical segments 4 mm in length. Each cylinder was set up for isometric recording in an organ bath according to the method described by NIELSEN and OWMAN (18). Briefly, the method consists in 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 a strain gauge for isometric recording. The latter pin is parallel to the former and movable, allowing the application of resting tension at right angles to the long axis of the vascular cylinder. The recording system included a Universal transducing Cell UC3, a Statham Micro-Scale Accessory UL5 and Beckman Type RS recorder. On either side of the vascular segment platinum electrodes were placed and the electrical stimuli were supplied by a Grass S4K stimulator. A resting tension of 1 g was applied to all preparations and readjusted every 15 minutes during an initial 100 minute equilibration period before cumulative frequency-response curves were made.

The organ bath contained 6 ml of Krebs-Henseleit solution at 37° C continuously bubbled with a 95 % oxygen: 5 % carbon dioxide mixture, which gave a pH of 7.3 to 7.4. 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; $NaHCO_3$, 25; glucose, 11.1; ethylenediamine tetracetic acid (EDTA, 3.10^{-5} M), was added to prevent oxidation of unstable substances. Drugs were dissolved in physiologic saline solution containing 0.01 % (w/v) ascorbic acid.

Trains of 300 square wave pulses of 0.5 msec. duration at 1, 2, 4, 8, 16 and 32 Hz were applied at supramaximal voltage. Frequency-response curves were determined in a cumulative manner, and control and experimental responses were obtained from separate vascular preparations. The drugs used to study their influence on the contractile response were added to the bath 10 minutes before electrical stimulation.

In 6 goats anesthetized with 2 percent sodium thiopental administered intravenously, both superior cervical sympathetic ganglia were exposed, isolated and removed under sterile conditions. These animals were killed 10-12 days postoperatively and prepared as the unoperated animals.

In 4 goats reserpine was administered

intravenously at a dose of 0.02 mg/kg/day for three days before they were killed.

The following drugs were used: reserpine (Ciba), phentolamine methanesulfonate (Ciba), cocaine hydrochloride (Abelló), tetrodotoxin (TTX) (Sankyo) and bretylium tosylate (Burroughs Wellcome). Statistical analysis was carried out by means of Student's «t» test, considering as significant a probability value of less than 5 %.

Results

In all the experiments, electrical stimulation of the communicating cerebral artery produced frequency-dependent increases in tension. The average at 32 Hz was 650 mg. When 3×10^{-6} M tetrodotoxin, an agent which blocks the conduction of nerve impulses, was present, the contraction induced by electrical stimulation was significantly reduced at all frequencies (fig. 1A). This fact indicates that part of the increase of tension is due to stimulation of the perivascular nerve endings.

In order to demonstrate pharmacologically that the vasoconstriction induced by electrical stimulation is due to the liberation of noradrenaline from adrenergic nerves present in the cerebral arteries, control vascular segments were electrically stimulated in presence of 10^{-6} M phentolamine, an α-adrenergic receptor blocking agent (fig. 1B), or 5×10^{-4} M bretylium, an adrenergic neuronal blocking agent (fig. 2A). Both reduced the contraction induced by electrical stimulation at all frecuencies. Nevertheless, when cocaine (10^{-6} M) was used to block the neuronal uptake of noradrenaline, there was no change in the frequency-response curve produced by electrical stimulation (fig. 2B).

The vasoconstrictor response induced by electrical stimulation of arterial segments from reserpinized goats and from goats on which 12 days earlier a bilateral superior cervical gangliectomy had been performed, was significantly reduced in both cases, as compared with controls (figure 3).

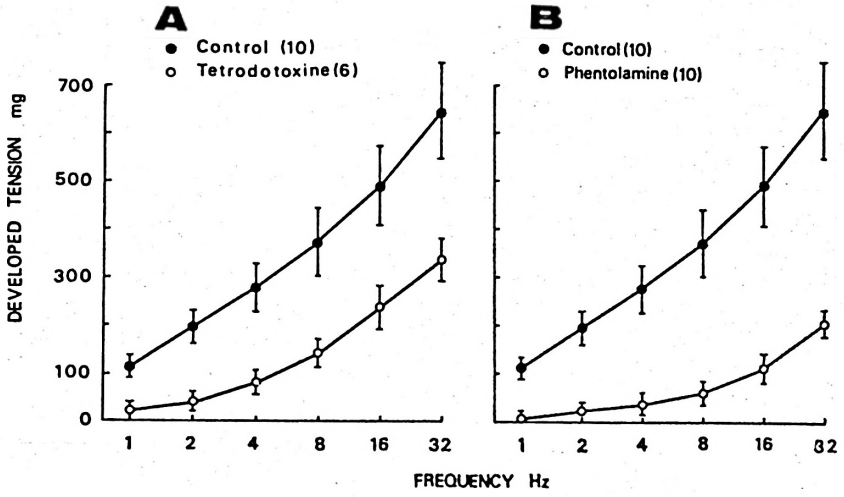

Fig. 1. *Frequency-response curves for field electrical stimulation of goat communicating cerebral artery of segments in the absence and presence of 3×10^{-6} M tetrodotoxin (TTX) (A) or 10^{-6} M phentolamine (B).*

Number of segments used to make each curve are indicated in parentheses. Vertical bars represent standard errors of the mean.

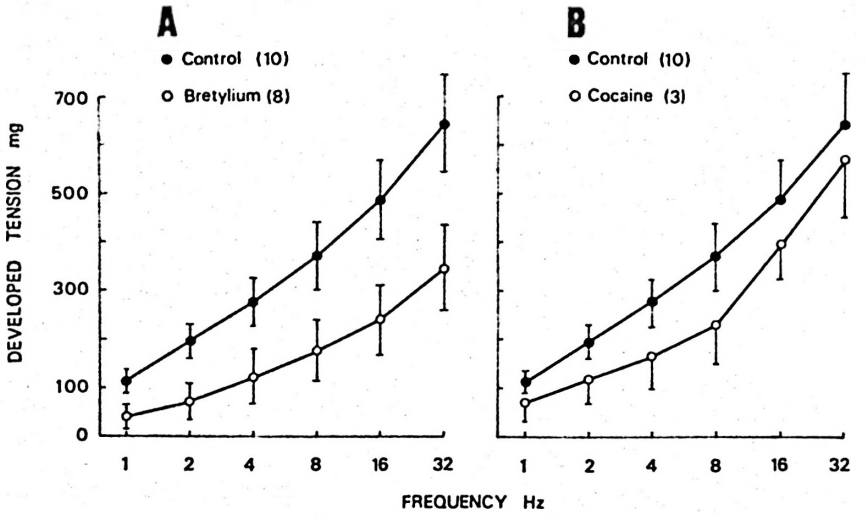

Fig. 2. *Frequency-response curves for field electrical stimulation of goat communicating cerebral artery segments in the absence and presence of* 5×10^{-4} *M bretylium (A) or* 10^{-4} *M cocaine (B).*
The other symbols are in figure 1.

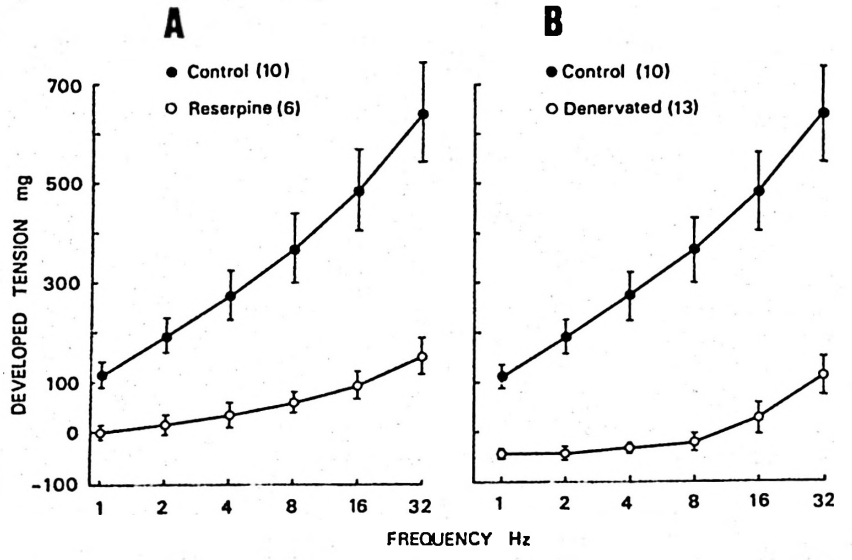

Fig. 3. *Frequency-response nerves for field electrical stimulation of communicating cerebral artery segments from reserpinized goats (A) and from goats in which both superior cervical sympathetic ganglia had been removed 12 days prior to the experiments (B).*
The other symbols are in figure 1.

Discussion

The present study shows that the electrical stimulation of the posterior communicating cerebral artery induced frequency-dependent vasoconstrictor responses. These results agree with those obtained in cerebral arteries of various animal species, including man (5, 8, 14, 16). However, these findings are not in accordance with those obtained by other authors in strips of bovine or dog brain arteries (4, 22). This fact led them to conclude that the inability of these arteries to contract was probably due either to the release of an insufficient amount of noradrenaline or sparse sympathetic innervation. Therefore, they inferred that this innervation probably does not play any important role in the control of the cerebral vascular tone. Perhaps the failure of these investigators to obtain contractile responses to electrical stimulation was due to the use of helical strips where the nerve endings might be more damaged because of the dissection than in the ring segments employed in the present study.

The vasoconstriction induced by field electrical stimulation on goat brain arteries was reduced in the presence of tetrodotoxin, an agent which blocks the conduction of nerve impulses, and bretylium, a drug interfering the release of the adrenergic neurotransmitter. This fact indicates that part of the tension increase is due to the stimulation of perivascular sympathetic nerve endings. These results agree with those obtained in other cerebral arteries (5, 14, 16). In addition, phentolamine, an alpha adrenergic blocking agent, reduced the contractile response induced by electrical stimulation. This finding suggests that this vasoconstriction is due to the release of noradrenaline from these tissues. These results are in agreement with observations *in vivo* in unanesthetized goats in which phentolamine significantly reduced the vasoconstriction induced by tyramine or electrical stimulation of cervical sympathetic nerves (15). Nevertheless, other authors have found that phentolamine augmented the vasoconstrictor response to electrical stimulation in rabbit basilar and dog cerebral arteries (15, 16).

On the other hand, the contractile response produced by electrical stimulation on the vessels from gangliectomized and reserpinized animals was also significantly less than that observed in the control ones. These findings give further support to the suggestion that this casoconstriction is mediated through an adrenergic mechanism. Similar results have been found in other brain arteries (5, 14). These facts agree with those obtained *in vivo* in unanesthetized goats in which the removal of cervical sympathetic ganglia increased the cerebral blood flow (1), and with the fact that reserpine pretreatment decreased the vasoconstriction induced by tyramine or electrical stimulation of the cervical sympathetic nerves (15).

When electrical stimulation was carried out in the presence of cocaine, an agent that blocks the neuronal uptake of amines, the contractile response was unaffected. In agreement with these findings are the results obtained in rabbit basilar (14), rabbit ear (3) and goat middle cerebral (5) arteries subjected to nerve stimulation in which cocaine showed a small and insignificant potentiation. Furthermore, cocaine did not potentiate the vasoconstriction produced by electrical stimulation of the cervical sympathetic nerves in unanesthetized goats either (15). It has been proposed that the potentiation by cocaine depends on the proximity between the adrenergic nerve terminals — responsible for the rapid uptake of noradrenaline — and the receptors in the effector cells (13).

In conclusion, field electrical stimulation of the posterior communicating cerebral artery of the goat produces a frequency-dependent contractile response which is partially antagonized by bretylium, TTX and phentolamine; the data indirectly but strongly suggest that the vascular contrac-

tile response induced by electrical field stimulation is partly due to the release of endogenous noradrenaline from perivascular sympathetic nerve endings.

Resumen

Se analiza la participación del sistema adrenérgico en la respuesta vasoconstrictora inducida por la estimulación eléctrica de campo en la arteria comunicante posterior aislada de cabra, utilizando para ello diversos procederes farmacológicos. Las arterias se sometieron a trenes de 300 impulsos (1-32 Hz, 0,5 msg) de voltaje supramaximal, lo que produjo en las mismas incrementos de tensión dependientes de la frecuencia utilizada. Estas respuestas vasoconstrictoras disminuyeron con tetrodotoxina (3×10^{-6} M), fentolamina (10^{-6} M) y bretilio (5×10^{-4} M); la cocaína (10^{-6} M) no las modificó. La contracción producida por la estimulación eléctrica en segmentos arteriales procedentes de cabras pretratadas con reserpina (0,02 mg/kg/día, durante 3 días) y de cabras a las que se les había extirpado ambos ganglios simpáticos cervicales superiores 12 días antes del experimento, se redujo significativamente con respecto a los controles. Estos resultados indican que la vasoconstricción inducida por la estimulación eléctrica de campo en las arterias cerebrales de cabra está mediada por un mecanismo adrenérgico.

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