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Changes in Mechanical Responsiveness of the Rat Aorta After Cervical Spinal Cord Stimulation

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Summary

The effect of cervical spinal cord stimulation on the mechanical vascular responsiveness of rat aortic artery rings was studied "in vitro". Arteries with and without endothelium from sham and stimulated male Wistar rats were placed in an organ bath studying the contractile response induced by noradrenaline, prostaglandine F2α and serotonin. For spinal cord stimulation two electrodes were placed in the cervical epidural space using microsurgical technique. The parameters of stimulation were: monophasic waveform current, pulse width of 0.1 ms, frequency of 120 cps, and intensity one third of the threshold which produced motor responses (between 0.3 and 0.5 v). The total period of stimulation was 120 minutes. The potency of each vaso-active agent was calculated in the organ bath for each experimental group. There were differences only when arteris with endothelium from sham (7.2587 ± 0.2308) and stimulated (8.0720 ± 0.3723) rats were tested with noradrenaline ($p < 0.01$).

Our results suggest that cervical spinal cord stimulation induces changes in the vascular wall that might explain the effects of spinal cord stimulation on vasomotor control.

Keywords: Spinal cord stimulation; vasomotor control; endothelium; derived contractile factor; aortic artery.

Introduction

Spinal cord stimulation (SCS) has been used for the treatment of ischaemic pain due to peripheral vascular disease. There are some clinical and experimental observations suggesting that SCS increases the peripheral blood flow[1, 5, 8, 9]. Although clinical indications of the SCS for peripheral vascular insufficiency seem to be properly delineated[1, 8], there are still some doubts about what are the mechanisms involved in this vascular effect. The purpose of this experimental work has been to study "in vitro" the mechanical responses of the aortic artery of rats subjected to SCS compared with that obtained from non stimulated animals, with the aim to elucidate the mechanisms of action on the vasomotor tone of the SCS.

Material and Methods

Animal Models

Wistar male rats, weighing between 200–250 g, have been used in the experiments. Animals were intraperitoneally anaesthetized with a mixture of ketamine chlorhydrate (100 mg/kg) and diazepam (2.5 mg/kg) and were maintained in spontaneous ventilation in a temperature controlled evironment. A group of sixty rats was subjected to cervical epidural spinal cord stimulation (Stimulation group). For this purpose a microsurgical C6 laminectomy was carried out and two electrodes were introduced into the epidural space. The electrodes were specially designed for this experiment and were 0.2 mm. in diameter and 2 mm. in length. Two electrodes were introduced cranially one on each side of the dorsal aspect of the dura mater. The parameters for the stimulation were: monophasic waveform current; pulse wavewidth of 0.1 msg.; frequency of 120 cps; intensity one third of the threshold which produced motor responses in the paraspinal muscles (between 0.3 and 0.5 v.); and, finally, a duration of stimulation of 120 minutes.

Another group of sixty animals was subjected to the same surgical procedures but stimulation was not carried out (Sham group).

The precise location of the electrodes was checked in each case by means of the observation of the areas of muscular response to stimulation and also by radiological control.

In four animals of each group a routine pathological study of the cervical spinal cord was undertaken, with the aim of demonstrating the lack of damage of the nervous tissues as a consequence of the surgical technique or the electrical stimulation.

Preparation of Arteries and Organ Bath Study

Animals were sacrified by sudden decapitation and exanguination just after the end of the stimulation period. In each case a portion of 10–15 mm. of thoracic aorta was obtained through a wide anterolateral thoracotomy. Artery isolation and removing took no more than 2 minutes. Arteries were quickly placed in a dissection

receptacle filled with a modified Krebs solution (milimolar composition: NaCl 120; KCl 4.5; MgCl 1.0; $NaHCO_3$ 27.0; KH_2PO_4 1.0; $CaCl_2$ 2.5; dextrose 10.0; ascorbic acid 0.1; and EDTA 0.04), constantly bubbled with a mixture of 95% oxygen (O_2) and 5% carbondioxide (CO_2). Arteries were handled always under homeostatic conditions. Under magnification adventicial layers were gently removed from the vessels wall in all cases.

In selected cases, sixty animals, the endothelium was mechanically destroyed by passing a guide through the vessel lumen. After that, ring portions of 5 mm. length were obtained from the center of each aorta to be studied in the organ bath. Each artery segment was suspended between two wire triangles in the bath. Bath receptacles contain a modified Krebs solution, bubbled with oxygen and CO_2 and maintained at 37 °C. The tension of the arteries was measured by means of an isotonic transducer* connected to an amplifier, and a graphic recording of each experiment was obtained. Four arteries were studied simultaneously, placing in each experiment two arteries from each experimental group. Arteries were left 45 minutes in the bath so as to stabilize the baseline vasomotor tone before the study with drugs. The contratension applied to the aorta segments was 2 g.

Noradrenaline inducing contractile responses of 14 aortic arteries obtained from normal unoperated rats were studied in the organ bath. The purpose of this study was to evaluate the responses of aortic artery in baseline conditions, validating the selection of this vessel.

Drugs

Noradrenaline ($5e^{-10}$ to $7e^{-4}$ M), serotonin ($2.5e^{-9}$ to $9e^{-4}$ M), and prostaglandin PGF-2α ($5e^{-10}$ to $1e^{-4}$ M) were used in the study#. All drugs were dissolved in bidistilled water except serotonin, which was diluted in 0.1 N HCl with 0.1% ascorbic acid. Drugs were added in equal volumes of 0.1 ml into the bottom of the bath.

Data Collection and Statistical Study

The dose-response curves obtained in the experiments had a sigmoid shape (Fig. 1). In each case the concentration that elicited the contraction (EC-0) and the concentration that produced the maximum response was calculated (EC-100). Then the concentration was determined that produced 50% of the maximum response (effective concentration 50%, EC-50) by means of a calculation of the regression line with several values of the dose-response curve taken between the 20 and 80% of the maximum response. For calculations, only dose-response curves with values of $r \geq 0.995$ were considered in the regression analysis. The potency (pD_2) of each drug was calculated as the negative logarithm of the EC-50 ($pD_2 = -\log$ EC-50).

Data were expressed as mean values ± standard deviation of the means. The potency of each drug in stimulated animals, both in arteries with and without endothelium, was statistically compared with Sham groups using a non-parametric test for means comparison (Mann-Whitney test). It was considered significant at a value of $p \leq 0.05$.

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Drugs obtained from Sigma Chemical Co, Poole, Dorset BH17 7NH, England.

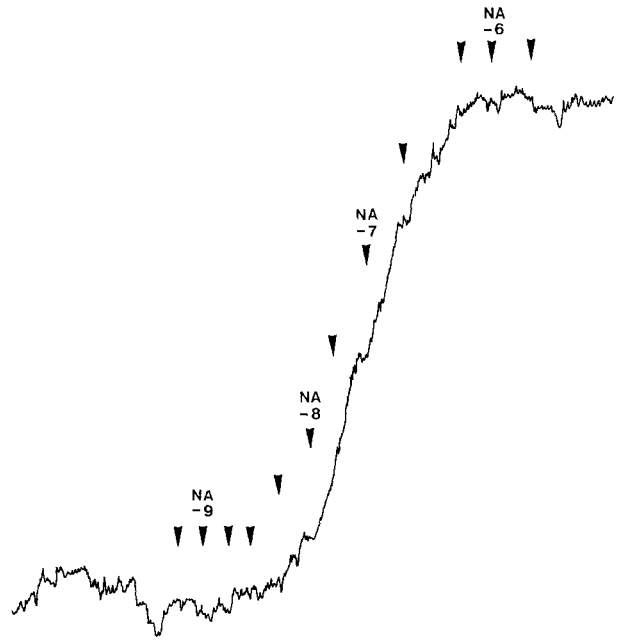

Fig. 1. Dose-response curve obtained with noradrenaline. In this case contraction started with $5e^{-9}$ M and the maximum was reached with $5e^{-7}$ M. The calculated EC-50 was $4.3e^{-8}$ M

Results

A valid dose-response curve was obtained using noradrenaline in normal aortic arteries. The potency of noradrenaline was 7.4645 ± 0.2950. The mean values and standard deviations of the potency of all experimental groups are showed in Table 1. Noradrenaline potency was significantly higher ($p < 0.01$) in aortas with endothelium from Stimulated animals than in Sham rats. When endothelium was destroyed the potency of noradrenaline decreases both in Stimulated and Sham aortas, but differences are not statistically different. Potency of serotonin and prostaglandin had no differences when they were tested on Stimulated or Sham arteries. In aortas without endothelium tested with serotonin or prostaglandin no valuable motor responses for drawing doseresponse curves were obtained.

Discussion

The effects of the SCS on peripheral vascular disease have been explained by means of at least one of the following mechanisms: analgesic action of the SCS which secondarily would interrupt the vasoconstrictor nociceptive reflexes; sympatholitic and vasodilator effect of the SCS by a segmentary or suprasegmentary action; and, finally, liberation of vaso-active substances

Table 1. *Potency of Noradrenaline, Serotonin, and Prostaglandin F2α in Stimulated and Sham Animals*

	With endothelium		Without endothelium	
	SCS	Sham	SCS	Sham
Noradrenaline	8.0720 ± 0.3723	7.2587 ± 0.2308*	6.7950 ± 0.7127	6.9914 ± 0.7543
Serotonin	5.3171 ± 0.5826	5.0819 ± 0.4336	nr	nr
Prostaglandin F2α	5.2247 ± 0.2313	5.1075 ± 0.1460	nr	nr

Values are means ± standard deviations of the means. * $p < 0.01$; nr = no valuable response. 10 arteries were tested in each group.

in the blood stream or directly in the vessel walls by SCS[1, 9]. Clinical studies have demonstrated by thermography that SCS produces an increase of the cutaneous temperature, an increase of the amplitude of the pulse wave by plethysmography and, therefore, an increase in the peripheral blood flow[1, 5, 8, 9]. Then, the analgesic effects of the SCS observed in patients with peripheral vascular disease would be secondary to these vascular changes. On the other hand, some clinical and experimental studies have shown that SCS produces only slight changes in the levels of biogene amines, neuropeptides and other peptides related to pain transmission or vasomotor regulation, both in blood and in cerebrospinal fluid[7, 10]. Moreover, when these changes are detected they have no relation with the levels of analgesia reached in the experiment. This lack of relationships between biochemical changes and clinical results could be explained if SCS would work inducing changes directly in the vessels wall and, therefore, with no significant biochemical modifications in blood or cerebrospinal fluid.

The lack of differences in the mechanical responses of the arteries tested with serotonin and prostaglandine PGF 2α does not allow us to draw conclusions about the possible role of these systems in the vasomotor action of the SCS. However, we have found that the vasomotor response of the vessel wall of the aortic artery against noradrenaline is different in Stimulated rats compared with Sham animals when they are studied "in vitro" in an organ bath. Although the aortic artery is more a conduit artery than a resistance vessel, the thoracic aorta has in rats a thick muscular layer and the contraction shown against nonspecific vasoactive agents is intense and dose-dependent. Therefore, aorta can be used to test our hypothesis studying its contractile responsiveness and mechanisms related to spinal cord stimulation.

Noradrenaline is more potent on arteries with endothelium in stimulated animals than in controls, probably due to an increase in the sensitivity of the vascular wall to the vasoconstrictor effect of the noradrenaline. A direct sympatholitic action of the SCS has been suggested by some authors[6], but there is no changes in bioamines in plasma or CSF[7, 10]. These facts suggest that SCS does not act directly on the sympathetic neurotransmitters. Probably SCS produces changes in some co-neurotransmitters or neuromodulators[3, 4]. By means of this indirect mechanism SCS would increase the percentage of free noradrenaline receptors and would decrease the basal vasomotor tone and thus increasing the noradrenaline potency in "in vitro" studies.

The effects of the SCS shown in our study are endothelium-dependent, since there were no differences between aortas Stimulated and Sham groups when endothelium was mechanically removed. The vasoconstrictor effect of the noradrenaline depends on the integrity of the endothelium. Nowadays, it is known that the endothelium is able to liberate vaso-active substances. The endothelial cell produce an "endothelium-derived relaxing factor" (EDRF), identified as nitric oxide, and also an "endothelium-derived constrictor factor" (EDCF), identified as a macromolecule called "endotheline"[11]. "In vitro" studies have shown that noradrenaline increases the levels of endotheline m-RNA, and therefore, the vasoconstrictor effects of the noradrenaline would be modulated by this factor. The lack of differences in the potency of noradrenaline in arteries without endothelium suggests the SCS effect would be blocking the action or the release of the endotheline mediated by the noradrenaline.

Although our experimental study has been done on arteries with no pathology, it is clear that in clinical situations SCS works on pathological vessels. Kanamuru *et al.*[2] have shown "in vitro" abnormal mechanical responses in artherosclerotic vessels. It is known that the endothelium or the artherosclerotic vessels is able to produce great amounts of vasoconstrictor

agents, specially endotheline. The hypothetized mechanism of action of SCS blocking the endotheline action would explain the effects of the SCS of pathological vessels. However, when the endothelium is lost or very injuried the action of the SCS would fail. This poor response in these situation was already pointed out by Meglio *et al.*[6]. On the other hand, SCS produces impressive improvements in frostbite patients, where the main pathophysiological events are vasoconstriction and sympathetic hyperactivity with no changes at the endothelium level.

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