Sympathetic Innervation of Cerebral Arteries: Prejunctional Supersensitivity to Norepinephrine After Sympathectomy or Cocaine Treatment

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Abstract: *Sympathetic Innervation of Cerebral Arteries: Prejunctional Supersensitivity to Norepinephrine After Sympathectomy or Cocaine Treatment*

■ The inactivation of the norepinephrine transmitter in the region of the adrenergic receptor is one important function of the sympathetic nerve terminals innervating blood vessels. This capacity was tested on isolated cat's middle cerebral artery (MCA) by recordings of the contractile response induced by norepinephrine at various stages after sympathectomy (excision of the superior cervical ganglion). Within three days after denervation, when fluorescence microscopy revealed a disappearance of neuronal norepinephrine in the vessel wall, there was a threefold increase in sensitivity of the test system which was not further enhanced at two weeks. This, and the finding of a similar amount of sensitization (of non-denervated vessels) to norepinephrine or tyramine after cocaine treatment, showed that a prejunctional type of sensitivity had developed. The sympathetic denervation did not influence the dose-response curve obtained with acetylcholine, supporting the specific nature of the supersensitivity reaction only to the sympathetic transmitter. Half a year after sympathectomy the sensitivity of the pial arteries to norepinephrine returned to control levels despite the absence of reinnervation, indicating that postjunctional changes also occurred. The findings offer further evidence for a functional role of the sympathetic nerves supplying intracranial arteries and show that the mode of innervation resembles that found in peripheral vessels.

Additional Key Words

cats | pial arteries | in vitro | tyramine | acetylcholine | fluorescence microscopy

Introduction

□ The adrenergic nerves of most vascular smooth muscles form a two-dimensional network superimposed on the media layer, which means that only a limited number of the muscle cells are in contact with the nerve terminals and hence become "directly innervated."[1-3] The same principal arrangement also is found in the pial vessels.[4-6] Here, the axon terminals are often located in furrows between two adjacent muscle cells which may give the impression of a penetration of nerves into the outer third of the vascular muscle layer.[7] Still, however, only the outermost layer of the smooth muscle cells is directly reached by the norepinephrine transmitter released upon nerve activation, as opposed to the situation where a three-dimensional plexus gives rise to nerve fibers that penetrate among the muscle cells of the media.[8, 9] Although Pease and Molinari[10] misinterpreted their findings on pial arteries as lack of innervation, they described the structural basis for a myogenic propagation of contraction in such vessels, following activation of the nerves at the surface of tunica media. For example, their findings of a special organization of myofibrillar attachments within the smooth muscle cells, and the shared basement membrane or even direct contacts between neighboring cells, are important features allowing for excitation spread throughout a given smooth muscular tissue.[11]

Studies concerning the mechanisms underlying the so-called supersensitivity of adrenoceptors to sympathomimetic amines[12] have contributed to the understanding of sympathetic neuroeffector systems. Because the nerve terminals constitute an important route of inactivation of norepinephrine in the region of the receptors,[13] these are reached by higher concentrations of the amine and hence the effect is potentiated when the neuronal uptake is abolished. This phenomenon of "prejunctional supersensitivity" can be demonstrated by postganglionic denervation or pretreatment with cocaine.[12] Such a pharmacological model was utilized to determine the ability of the perivascular sympathetic nerves[4] to inactivate the sympathetic transmitter, norepinephrine, in the region

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of the adrenergic receptors present in the pial arterial wall.[14]

Methods

ANIMALS

Fifty-three cats of either sex, weighing 2 to 5 kg, were used. The pial arteries were sympathectomized unilaterally or bilaterally in 30 animals by removal (through a midline neck incision under pentobarbital anesthesia) of the superior cervical ganglia, the origin for all adrenergic nerves supplying the middle cerebral arteries (MCAs)[4] which were used in the tests. The animals were exsanguinated and decapitated under light pentobarbital anesthesia at various time intervals after denervation. The brains from denervated as well as unoperated animals were immediately removed and a 5-mm portion of the proximal part of the MCAs was carefully dissected out.

IN VITRO SYSTEM

Pieces of the MCAs from both sides were mounted between two separate systems of L-shaped metal holders in the same 50-ml mantled organ bath containing a Krebs-Ringer buffer solution of the following composition (mM concentration): NaCl 118; KCl 4.5; $CaCl_2$, $2H_2O$ 2.5; $MgSO_4$, $7H_2O$ 1.0; $NaHCO_3$ 25; KH_2PO_4 1.0; glucose 6.0. It was thermostatically maintained at 37.5°C (range ± 0.2°C), measured with a needle thermocouple placed near the blood vessels. A mixture of 95% O_2 and 5% CO_2 was permanently bubbled through both the bath and the connected stock solution, which was also kept at 37.5°C. The rate of aeration was chosen to give a pH in the bath of 7.380 ± 0.005 (mean ± SEM). This was measured in 50 µl samples taken from the bath, using a pH Meter 27 with type E 5021 electrode unit (Radiometer, Copenhagen). Isometric circular tension was recorded by force-displacement transducers (Endevco Model 8107-2) and monitored on a Grass Model 7B polygraph. The vessels were given an initial load of 400 dynes after which they stabilized at a steady level of tension about 50 dynes lower. Tests were carried out following a two-hour accommodation time. For further methodological considerations, see Edvinsson et al.[15] The dose-response curves were obtained by cumulative application of the agonist. Sensitivity of the test system was expressed in terms of ED_{50} values (concentration of agonist at which half maximum response occurs). Mean values were compared statistically according to Student's *t*-test.

MICROSCOPY

Those pial arteries at the base of the brain not used in the pharmacological tests were dissected out, cut open longitudinally and mounted flat on microscope slides, dried over phosphorus pentoxide for one hour and exposed to formaldehyde gas for one hour for fluorescence microscopic demonstration of adrenergic (sympathetic) nerves.[4]

DRUGS

The drugs used in this study were: *L*-norepinephrine hydrochloride (*L*-arterenol hydrochloride; Sigma), acetylcholine hydrochloride (Calbiochem), tyramine hydrochloride (Sigma), cocaine hydrochloride (ACO, Sweden). In order to avoid interference from the non-neuronal uptake of norepinephrine,[13] *DL*-normetanephrine hydrochloride (Sigma) was present in the bath at a concentration of 10^{-6}M throughout the test. Since norepinephrine stimulates both the pial arterial alpha-receptors and beta-receptors,[14] but only the contractile response was recorded in the tests, the beta-receptors were maintained inhibited by 10^{-9}M propranolol chloride (Inderal, Scanmeda) in the buffer stock solution.

The substances were dissolved in 0.9% saline which, in the case of norepinephrine, contained 0.2 mg per milliliter of ascorbic acid to minimize oxidation. Concentrations cited below are given as the salt, and expressed as the final molar concentration in the bath.

Results

Pial arteries from normal and ganglionectomized cats were compared with regard to the contractile effects of graded concentrations of norepinephrine tested after varying time intervals following operation. The dilatory response, mediated by the beta-receptors,[14] was inhibited by the presence of propranolol, and any non-neural uptake of the amine[13] was blocked by normetanephrine present in the bath. When sympathectomy was unilateral,[4] the arteries from the intact and denervated side were run together in the same test system. The mean ED_{50} value for non-denervated arteries was $(1.95 \pm 0.29) \times 10^{-6}$M (± SEM). Shortly after denervation the sensitivity of the test system to norepinephrine tended to decrease [$ED_{50} = (3.88 \pm 1.88) \times 10^{-6}$M] although the difference in comparison with the intact vessels was nonsignificant. At three days following the sympathectomy (fig. 1), a pronounced and significant increase in sensitivity had developed [$ED_{50} = (0.77 \pm 0.19) \times 10^{-6}$M]. A similar degree of supersensitivity was found also at one week and two weeks postoperatively, with mean ED_{50} values of $(0.77 \pm 0.46) \times 10^{-6}$M and $(0.60 \pm 0.13) \times 10^{-6}$M, respectively (fig. 1). Representative dose-response relations for a non-denervated vessel and an artery sympathectomized two weeks previously are illustrated in figure 2. After half a year the ED_{50} values had normalized to levels [$(2.52 \pm 0.38) \times 10^{-6}$M] not significantly different from those of the non-denervated arteries.

As revealed by fluorescence histochemistry, all pial arteries at the base of the brain are supplied by adrenergic nerves to a varying extent: vessels emanating from the carotid system have a more pronounced innervation than those belonging to the vertebral system.[4] There was a slight reduction in the fluorescence of the nerve plexuses at one day postoperatively, whereas from three days onward no fluorescent fibers were visible around the vessels. Fluorescent microscopy of preparations taken six months after bilateral sympathectomy showed no signs of adrenergic reinnervation.

Administration of cocaine (10^{-6}M) to the bath 20 minutes before testing non-denervated arteries gave a mean ED_{50} value of $(0.59 \pm 0.14) \times 10^{-6}$M, which was in the same order of magnitude as obtained at about one to two weeks following sympathectomy (fig. 1). Equal ED_{50} values were obtained for norepinephrine

tested on vessels six months after sympathectomy whether cocaine was present in the bath or not. In a few experiments (performed on the non-denervated arteries) with cocaine and norepinephrine in the presence or absence of normetanephrine (fig. 3a), it was shown that the presumed non-neural uptake process[15] is of little or no significance in removing norepinephrine from the region of the pial vascular adrenoceptors under the conditions studied.

Experiments on innervated middle cerebral arteries also were carried out with tyramine,[16] whose specific sympathomimetic effect is exerted via an uptake into the adrenergic nerves followed by displacement and release of endogenous norepinephrine onto the adrenergic receptors. Cocaine pretreatment caused an increase in sensitivity also to this indirectly acting amine (fig. 3b).

In order to check that sympathetic denervation of the pial artery produced a specific supersensitivity not involving, e.g., the cholinergic transmitter, a series of experiments was performed with acetylcholine. This agonist has a dual action on pial arteries: at low doses it dilates and at higher doses it contracts the vessel, and both effects are blocked competitively by atropine.[17] Dose-response curves from the contractile response of sympathectomized arteries did not show any parallel shift as compared with controls (fig. 4).

Discussion

The pial vascular system in a variety of mammals,[4, 18] including man,[19] is supplied by a very dense system of sympathetic nerve terminals; in fact, the innervation in some of the regions is even better developed than in such well-innervated vessels as the mesenteric arteries. In the adventitia-medial border the nerve terminals form a two-dimensional plexus approaching the membranes of the outermost smooth muscle cells of, for example, the MCA, used as the model in the present study, by approximately 80 to 100 nm.[5-7] All sympathetic — but none of the cholinergic[18, 20] — nerves in this and other arteries at the base of the brain originate in the superior cervical ganglia.[4] The norepinephrine transmitter disappears within three days after excision of the ganglia,[20, 21] each of which has a strictly unilateral contribution.[4] Thus, after denervating the artery on one side, that of the contralateral side can be used as control.

A sensitive method[15] for pharmacological analysis of the circular vasomotor response in isolated small vessels (200 μ inner diameter) has recently been

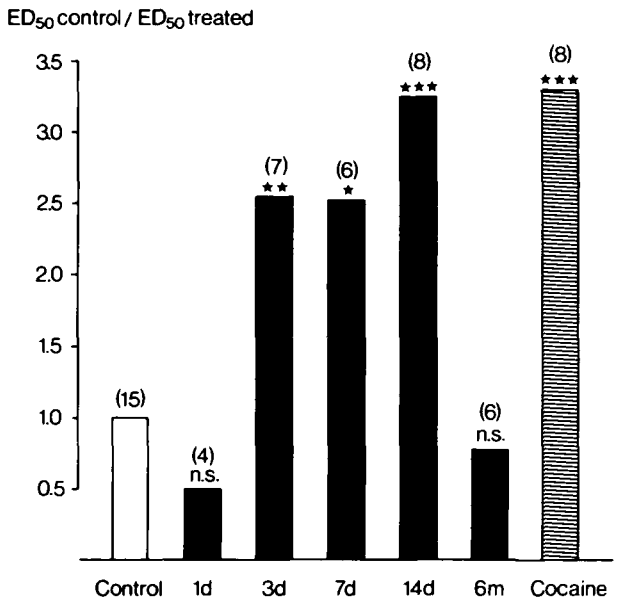

FIGURE 1

*Effect of sympathectomy (one day to six months; filled bars) and 10^{-6} M cocaine (hatched bar) on the norepinephrine-induced contraction of MCAs as compared with non-denervated control vessels (open bar). Full cumulative dose-response curves (see figs. 2 and 3) were run, and mean value compared for the norepinephrine doses giving half maximum response (ED_{50}). The bath contained 10^{-9}M propranolol and 10^{-6}M normetanephrine. Comparison of mean ED_{50} values according to Student's t test (the groups consisted of unilateral denervations with contralateral control, bilateral denervations, and control vessels from untreated animals): *0.01 < P < 0.05; **0.001 < P < 0.01; *** P < 0.001, n.s. = not significant. Number of tests within parentheses.*

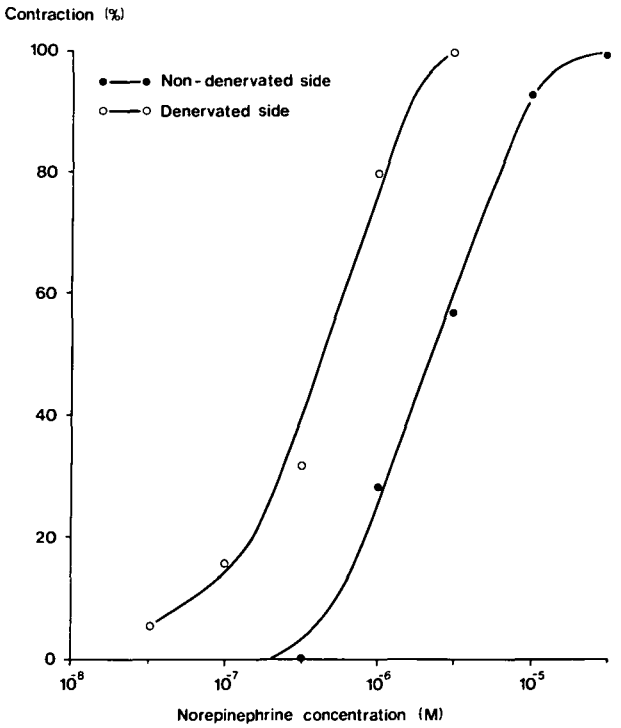

FIGURE 2

Representative examples of cumulative dose-response curves for norepinephrine-induced contraction of pial vessels from a cat in which the superior cervical ganglion was removed on one side two weeks before the tests. Blocking agents as in figure 1. Ordinate shows relative contraction with maximum response for each test series set as 100%.

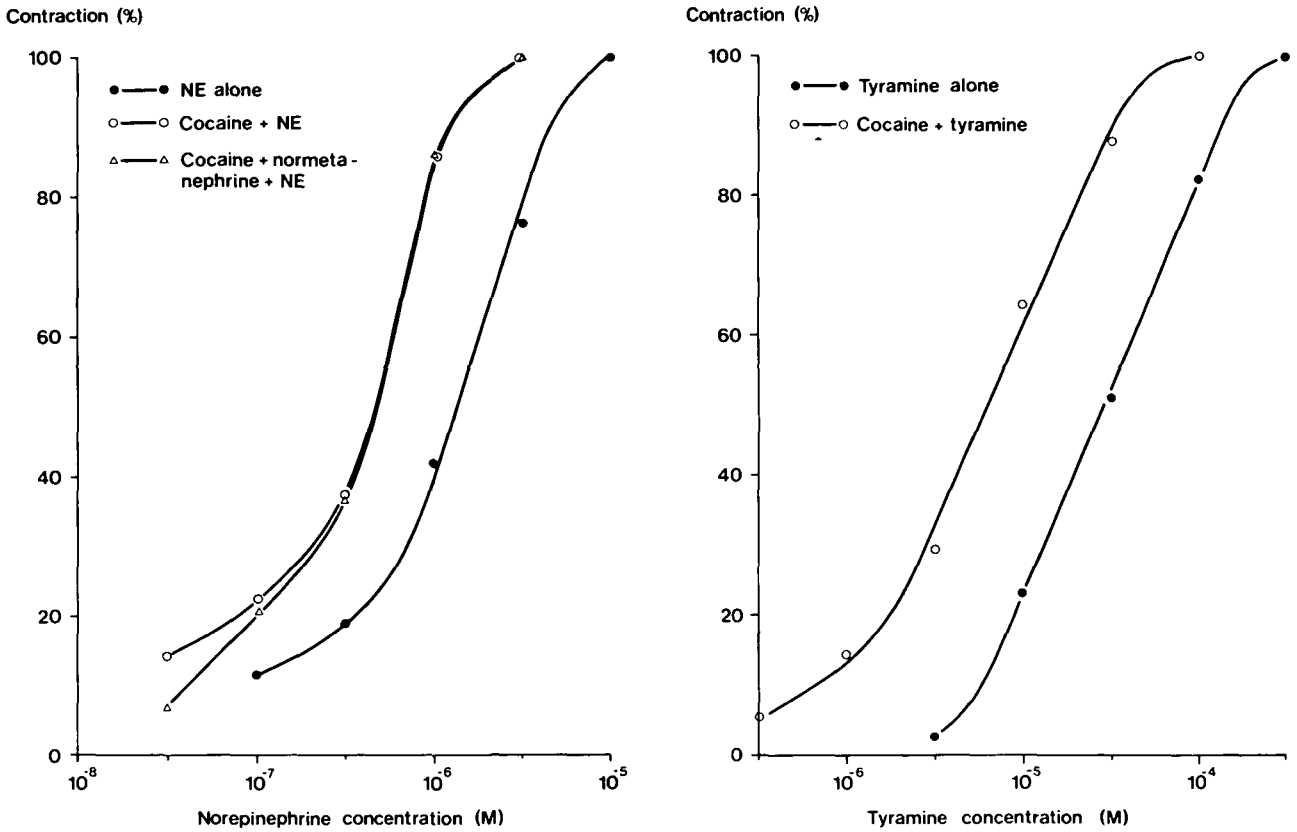

FIGURE 3

Tests on non-denervated vessels (in the presence of $10^{-9}M$ propranolol). (a) There is a clear increase in sensitivity of the response to norepinephrine after administration of cocaine ($10^{-6}M$), but no further effect by addition of normetanephrine ($10^{-6}M$). (b) Also, tyramine contracts the pial artery and the sensitivity of the test system increases in the presence of cocaine ($10^{-6}M$). In addition to propranolol the bath contained $10^{-6}M$ normetanephrine.

used for characterization of the adrenoceptors in feline MCA.[14] The alpha-receptors, mediating vasoconstriction, showed certain features different from those in the peripheral circulation: only partial agonism with phenylephrine (which also acted on sites other than the alpha-receptors), an unusually high ED_{50} for various sympathomimetic amines and, for certain antagonists, different dissociation constants compared with those obtained for other smooth muscle tissues. The beta-receptors, mediating vasodilation, turned out to be of the $beta_1$-type whereas peripheral vascular beds, with exception of the coronary circulation, seem to possess $beta_2$-receptors.

Complete sympathetic denervation of the MCA produced a threefold increase in sensitivity of the test system to exogenous norepinephrine. This denervation supersensitivity is of the prejunctional type[12] because it (a) was fully developed shortly after the operation, coinciding with the degeneration of the pial perivascular sympathetic nerves,[20] and (b) could be revealed in the innervated arterial preparations by administration of cocaine,[22] which blocks the neuronal uptake of norepinephrine. The prejunctional supersensitivity is, in fact, not a true increase in receptor sensitivity but an enhanced reactivity of the test system: this becomes exposed to higher norepinephrine levels after elimination of the neuronal uptake mechanism, which normally removes 70% of norepinephrine in the region of the receptors.[23] Addition of normetanephrine[13] did not produce a further parallel shift to the left of the norepinephrine dose-response curve. This is in agreement with observations showing that the non-neuronal (i.e., muscular) uptake of transmitter is considerably lower than the neuronal uptake.[23]

In an attempt to show that pial vasomotor nerves are able to release the norepinephrine transmitter in amounts sufficient to produce a vasoconstriction, experiments were carried out to show that tyramine contracted the MCA provided the perivascular plexus was intact.[16] This functional role of the sympathetic nerves in the brain vascular bed was confirmed by tyramine experiments in vivo.[24] It also was confirmed on isolated pial vessels by electric field-stimulation of the perivascular sympathetic nerves.[25] The above-mentioned tyramine experiments performed on the feline MCA have recently been repeated to some extent on the same artery from goats by Urquilla et al.[26]

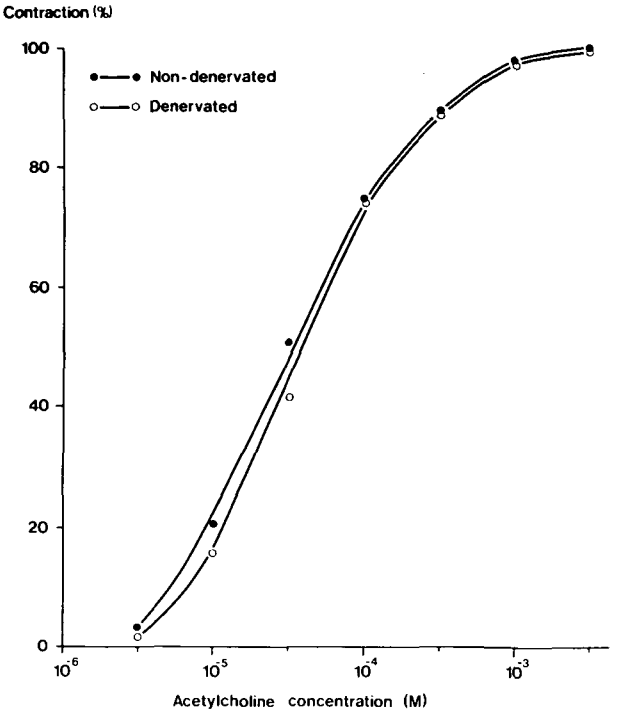

FIGURE 4

Unilateral denervation (seven days) of the pial artery does not alter the response to acetylcholine as compared with that of the intact vessel from the contralateral side.

and the conclusions drawn agree with those previously presented by us.[16] The effect of cocaine on the dose-response curve of tyramine is complex.[27] In the present study, pretreatment with 10^{-6}M cocaine sensitized the vascular preparation to small amounts (below 5×10^{-5}M) of tyramine, which may be caused by pronounced supersensitivity to released (endogenous) norepinephrine combined with a reduced effectivity of tyramine.[27] The neuronal uptake of tyramine becomes inhibited to a large extent by higher concentrations of cocaine and its effect therefore is diminished, overcoming the sensitization to norepinephrine.[27] This would be in accordance with the subsensitivity of the MCA to tyramine shown by Urquilla et al.[26] at higher concentrations of cocaine (10^{-4}M) and larger doses of tyramine (above 5×10^{-5}M).

There was not any further significant increase in sensitivity to norepinephrine after the first three days of denervation, and the response to acetylcholine was not altered, indicating that no unspecific postjunctional supersensitivity had developed. However, when tested half a year after sympathectomy, the sensitivity of the vessel to norepinephrine had returned to the same level as in non-denervated arteries, although sympathetic reinnervation had not occurred. This, and the failure of cocaine to potentiate the response, indicates that compensatory postjunctional changes of the receptor mechanism may take place during prolonged denervation.

As reviewed by Trendelenburg,[28] it has been suggested that there is a relation between the organization and degree of sympathetic innervation of a smooth muscular tissue and the amount of prejunctional supersensitivity produced by denervation or cocaine treatment. Thus, sensitivity to norepinephrine increases 10-fold to 20-fold in tissues such as the portal vein, nictitating membrane, and vas deferens, which all receive a well-developed three-dimensional system of sympathetic nerve terminals.[8, 29, 30] In arterial preparations — including the pial artery tested in the present study — where only the outermost layer of smooth muscle cells are reached by the nerve terminals,[3, 4] there is only about three times of increase in sensitivity.[31] With increasing length of minimal neuromuscular distance the degree of supersensitivity is lower as tested with norepinephrine after pretreatment with cocaine.[32] In pial arteries this distance is 80 to 100 nm[5-7] and our finding of a threefold increase in sensitivity is in good agreement with the relationship between sensitivity and neuromuscular interval shown by Verity.[32]

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