Evidence That Vasoactive Intestinal Polypeptide (VIP) Mediates Neurogenic Vasodilation of Feline Cerebral Arteries

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SUMMARY In this study the magnitude of non-sympathetic, non-cholinergic neurogenic vasodilation of feline cerebral arteries in vitro was correlated with the extent of innervation by VIP-immunoreactive nerves. Well-innervated arteries underwent nerve-mediated relaxation whereas those that are not supplied with VIP-containing axons did not relax to transmural nerve stimulation. The relaxation of cerebral arteries that are well endowed with VIP-immunoreactive nerves was selectively and reversibly inhibited by VIP-specific antiserum. Substance P-specific antiserum did not affect the dilator responses. We conclude that VIP is a functional neuromediator transmitter in the cerebral circulation.

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ALTHOUGH VIP was first isolated from porcine small intestine and classified as a hormone,1-2 VIP-like immunoreactivity was soon discovered within nerves that distribute to a number of organ systems.3-5 In 1976 Larsson and his colleagues6 described perivascular nerves that were immunoreactive for VIP within the larger cerebral blood vessel that is richly endowed with VIP-IR nerves. Our findings support and confirm the previous suggestions of a specific vasodilator transmitter role for VIP-containing neurons within the cerebral circulation. First, using in vitro techniques, we have studied non-cholinergic dilator responses of feline cerebral arteries that receive differing degrees of innervation by VIP-IR nerves, from dense to none, to elucidate possible correlations between innervation and physiological response. In addition, we have assessed the inhibitory actions of VIP-specific antiserum on non-adrenergic, non-cholinergic neurogenic vasodilator responses of the feline middle cerebral artery, a blood vessel that is richly endowed with VIP-IR nerves. Our findings support and confirm the previous suggestions of a specific vasodilator transmitter role for VIP in the cerebral circulation.

Methods

Adult, mongrel cats of either sex and weighing 4-5 kg were anesthetized with sodium pentobarbital and exsanguinated. The brain was quickly removed and placed in Krebs bicarbonate buffer that was gassed with 95% O₂ and 5% CO₂ (pH 7.4). In a first series of experiments, the anterior inferior cerebellar artery and a second order branch of the middle cerebral artery (lumen diameter: ~0.3 mm) were dissected from the brain and placed in Krebs solution. The cerebellar artery was separated into two segments, one proximal (diameter: ~0.3 mm) and one distal (diameter: ~0.25 mm) to the first major side branch of the artery. Cylindrical segments of these arteries (3 mm in length) were
mounted between two wires, one of which was attached to an isometric force transducer and the other to a micrometer displacement device. Samples were then immersed in Krebs bicarbonate buffer contained in an organ bath (3.0 ml volume) and were maintained at 37°C and pH 7.4. Resting tone was adjusted to 0.1 g and after the preparations had equilibrated for 1 hour, active tone was induced using prostaglandin F_2α (3 × 10^{-6}M). Vasodilator responses to transmural nerve stimulation (TNS) (0.3 msec pulses, 170 milliamps) and to exogenous VIP were then measured. TNS was delivered through platinum electrodes positioned one on either side of the artery and exogenous VIP was added directly to the organ bath. Relaxations were observed in the presence of guanethidine (5 × 10^{-6}M) and to exogenous VIP were then measured. TNS was confirmed in each experiment by exposing the preparations to tetrodotoxin (TTX) (3 × 10^{-7} M) for 10 minutes and checking for abolition of the electrically evoked vasodilations. At the completion of the organ bath experiments, each arterial segment was examined for the presence of VIP-IR nerves using immunofluorescence histochemistry as described previously. In experiments designed to study the effects of VIP-antiserum, a slightly different protocol was followed. Proximal segments of the middle cerebral artery (lumen diameter: =0.45 mm) were isolated and tested as described above for their dilator responses to TNS, exogenous VIP and, in addition, to NaNO_2. Arterial samples were then washed several times and incubated in a given amount (0, 12.5, 25, or 50 microliters) of VIP-specific antiserum. The antiserum stock was prepared by dissolving a lyophylized sample in 1 liter of VIP-specific antiserum. The antiserum stock was not cross-reactive (manufacturer’s specifications) with related peptides including secretin, glucagon, peptide HI or gastric inhibitory peptide. In these experiments we anticipated difficulties regarding foaming of the antiserum-containing solutions when gassed with O_2/CO_2. However, we found that pH could be maintained in the range of 7.3–7.4 in the small volume organ baths without excessive foaming using very slow rates of bubbling. After two hours incubation with the antiserum, arteries were activated for a second time and dilations in response to TNS, exogenous VIP and nitrite, in the presence of antiserum, were examined. The antiserum was subsequently removed by washing every ten minutes for a period of one hour, and then vasodilator responses were studied once again. In three experiments the effects of antiserum specific for substance P were tested in a manner and in amounts identical to that described above for the VIP-antiserum. The following drugs were used: prostaglandin F_2α (Sigma), guanethidine sulfate (Broughs Wellcome), atropine sulfate (Sigma), TTX (Sigma), substance P-antiserum (Cambridge Research Biochemicals, Ltd.), VIP-antiserum (Cambridge Research Biochemicals, Ltd., lot 17094) and VIP (Peninsula Laboratories). Data are expressed as mean ± S.E. and some data were analyzed for statistical differences by analysis of variance.

**Results**

These studies revealed a clear association between atropine-resistant neurogenic vasodilation and the extent of innervation by VIP-IR nerves. A branch of the middle cerebral artery that is well-innervated underwent tetrodotoxin-sensitive, frequency-dependent vasodilations which were mimicked by exogenous VIP (fig. 1). A more modestly innervated segment of the anterior inferior cerebellar artery also relaxed during TNS and to exogenous VIP but to a lesser extent than that of the middle cerebral artery. A non-innervated portion of the anterior inferior cerebellar artery did not relax to TNS, although slight relaxations to exogenous VIP were observed. The findings that cerebral arteries supplied with VIP-IR nerves relax to TNS and those that do not receive such nerves do not relax to TNS was confirmed in middle cerebral and cerebellar arteries taken from five animals.

The involvement of VIP in the dilator responses was demonstrated further in experiments on the well-innervated middle cerebral artery. The inhibitory effects of different amounts of VIP antiserum on neurogenic relaxations of middle cerebral arteries are shown in figure 2. Responses of untreated preparations did not change with time whereas preparations treated with increasing amounts of antiserum showed a progressive decrease in relaxation that returned to pre-treatment values following washout of the antiserum. Treatment of the tissues with antiserum did not influence the levels of PGF_2α-induced tone. For instance, in the presence of 50 microliters VIP-antiserum arteries developed 0.38 ± 0.06 grams force; pre- and posttreatment

![Figure 1. Responses of cerebral arteries to transmural nerve stimulation and exogenous VIP. Tone was induced by PGF_2α (3 × 10^{-9}M) in the presence of guanethidine (5 × 10^{-6}M) and atropine (3 × 10^{-7}M). Drug doses at arrows are indicated as negative log molar concentrations. MC: middle cerebral artery; AIC: anterior inferior cerebellar artery. Innervation density: +++, dense innervation; +, modest innervation; 0, no VIP-immunoreactive nerves.](https://example.com/figure1.png)
Responses to PGF2α were 0.44 ± 0.04 g and 0.40 ± 0.06 g, respectively.

The effects of VIP-antiserum on relaxations to 2, 4 and 8 Hz stimulation and to exogenous VIP and the effects of substance P-specific antiserum on relaxations to 8 Hz stimulation are shown in figure 3. At the highest dose of VIP-antiserum the responses to nerve stimulation at 2 and 4 Hz were abolished and responses to 8 Hz and to exogenous VIP were greatly reduced. Dilation to NaNO2 (10^-4M) was not different before and during treatment with VIP-antiserum. In all cases nitrite caused relaxation of greater than 60% of the induced tone. Treatment of the arteries with increasing amounts of substance P-specific antiserum had no effect on responses to TNS (fig. 3) or to exogenous VIP (data not shown).

Discussion

When considered together, recent experimental evidence, including that presented in the present paper, indicates a vasodilator transmitter role for VIP in the cerebral circulation. For instance, using the immunogold technique, Lee et al16 found VIP-IR material within some large granular vesicles in non-sympathetic nerve terminals in the wall of feline cerebral arteries.

Release of VIP from cerebral arteries in vitro following TNS may or after application of high concentrations of potassium has been observed. Nanomolar to micromolar amounts of VIP induce vasodilation of feline cerebral arteries both in vitro and in vivo. Similar latencies for onset of relaxation and similar half-times for recovery of tone following TNS or addition of low concentrations of VIP have been demonstrated in the feline middle cerebral artery. The present report of a link between degree of innervation by VIP-immunoreactive axons and extent of atropine-resistant vasodilation and of an inhibitory effect of VIP-specific antiserum on nerve-mediated and VIP-induced relaxations of middle cerebral arteries further implicates VIP as a transmitter in the cerebral circulation. The relationship between degree of innervation and response to nerve stimulation in this study is similar to that reported for mesenteric arteries by Furness and Marshall where density of innervation by adrenergic nerves and constrictor response to nerve stimulation were compared. These authors found that well-innervated arteries constricted to nerve stimulation whereas non-innervated arterioles did not respond. Griffith et al also reported correlations between extent of adrenergic innervation, noradrenaline content and constrictor response to nerve stimulation in the rabbit central ear artery. Similar studies for dilator systems have not been reported.

The dilator response of the distal anterior inferior cerebellar artery to exogenous VIP suggests that receptors for VIP extend into regions of the vasculature that do not receive VIP-IR nerves, at least within the cerebral circulation. This probably is not a general phenomenon. For instance, the posterior auricular artery in the cat, an artery where neurogenic dilation is a purely cholinergic event, does not receive VIP-IR nerves and does not dilate to exogenous VIP (unpublished observation).

Others have reported inhibitory effects of VIP-antiserum on neurogenic vasodilation. Lundberg observed a decreased dilator response versus control during stimulation of the autonomic innervation of the submandibular salivary gland when VIP-antiserum was present. Bevan et al reported an inhibitory effect of VIP-antiserum on dilator responses of the feline lingual artery and Goadsby and MacDonald observed an attenuation of neurogenic dilation in the feline extracerebral circulation in vivo in the presence of VIP-antiserum. VIP-antiserum also reduced electrically evoked vasodilation of feline pial arteries in situ as well as neurogenic inhibitory responses thought to be mediated by VIP in non-vascular smooth muscles. The specific mechanism of action of the antiserum in these experiments is unknown. The most likely explanation is that VIP released from nerves is bound by the antiserum before the VIP can act on post-synaptic vascular receptors. The lack of effect of substance P-specific antiserum on neurogenic and VIP-induced dilations and lack of effect of VIP-antiserum on dilations to nitrite argue against a non-specific action of the VIP-antiserum.
Although neurogenic vasodilations were very nearly abolished by high concentrations of VIP-antiserum in these experiments, we cannot rule out the possibility that other non-adrenergic, non-cholinergic transmitters may mediate cerebral vasodilations in some instances. A portion (15–20%) of the total neurogenic dilator response at 8 Hz was not blocked by the antiserum and higher frequencies of stimulation or application of current intensities greater than those used in the present study might have revealed other components of the dilator responses. Vasodilator peptides such as peptide HI\(^2\) and calcitonin gene-related peptide\(^2\) have been observed in cerebrovascular perivascular nerves and these substances warrant future consideration as possible vasodilator transmitters in the cerebral circulation.

In summary, we have presented data that indicate that the magnitude of neurogenic vasodilation in feline cerebral arteries is proportional to the extent of innervation by VIP-IR nerves. In addition, VIP-antiserum can inhibit specifically the dilator responses to TNS and to exogenous VIP. We conclude that VIP-containing nerves represent a functional innervation of cerebral arteries. The widespread distribution of VIP-IR nerves within the cerebral circulation of many species suggests that this neuronal system could play an important role in control of cerebral blood flow.

References
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