Sympathetic, muscarinic vasodilation in cranial vessels of the cat

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Summary. Pressor responses evoked by stimulation of the preganglionic sympathetic trunk of the feline superior cervical ganglion have been recorded in vivo from the vascular bed perfused by one external carotid and the vertebral artery. When vasoconstrictor activity is blocked and potential vasodilator activity enhanced by close, intracarotid injection of guanethidine and prostaglandin $F_{2\alpha}$ respectively, stimulation evokes a weak pressor response followed, on cessation of stimulation, by a prolonged vasodilation lasting for 6-8 min. The magnitude and duration of the poststimulation vasodilation was reduced significantly by atropine. Due to the prolonged nature of the vasodilation, it is unlikely that a sympathetic cholinergic vasodilation in the classical sense is involved.
Key words. Neurogenic vasodilation; muscarinic receptors; superior cervical ganglion.

A vascular model useful for monitoring the efficiency of transmission of vasomotor information through the superior cervical ganglion (SCG) has been recently described[1]. In comparison to the tension developed by the nictitating membrane (NMT) during stimulation of the SCG, the cranial pressor responses (CP) appeared to be more dependent upon the stimulus conditions and more sensitive to metoprolol than did NMT. These studies also showed that mechanical distension of the carotid sinus, denervated except for the Herring nerve, facilitated CP but not NMT. This suggested to us that CP could also be used to illustrate the effects of integration of peripheral afferent with vasomotor efferent synapses within the SCG. Facilitation of CP by peripheral afferents could occur either by their enhancing the transmission of vasoconstrictor activity through the SCG, or by their inhibition of cholinergic transmission. The latter was initially rejected since atropine was ineffective in enhancing CP, but this possibility was not exhausted since an overwhelming pressor effector response might mask a weaker vasodilator response. We were encouraged in this possibility by a number of studies which have shown that segments of cranial and cerebral vessles in vitro respond to transmural nerve stimulation by a biphasic relaxation, a portion of which is abolished by atropine[2-4]. In order to demonstrate this, it was first necessary to block contraction with guanethidine, and then to restore tone with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Once accomplished, an initial relaxation was observed during transmural nerve stimulation. This was blocked by atropine. A second, delayed relaxation to stimulation was insensitive to atropine. This persisted for a number of minutes. The mediator was not identified. Other investigators have detected similar prolonged noncholinergic vasodilator responses in the cat hind limb after stimulation of the sympathetic nerves[5-8]. Histochemical evidence supporting cholinergic activity has been found at the level of the cranial and cerebral vessels[4,9]. Ablation studies seem to indicate that these enzyme stores were not under the control of sympathetic nerves originating from the SCG[4,10].

We determined to see if our model of cranial circulation and sympathetic innervation could be used to demonstrate a cholinergic vasodilator response. Five male cats weighing 3–4 kg were anesthetized with pentobarbital (30 mg/kg i.p.) and prepared as previously described for recording NMT and CP[1]. Briefly, cranial pressure was recorded from right external carotid artery. The left carotid and vertebral arteries were left intact. Stimulation of the preganglionic trunk supplying the SCG on the right served to develop NMT and increase CP. The right vagus, carotid sinus and depressor nerves were cut.

Using CP as a guide, the sympathetic preganglionic trunk on the right was stimulated at increasing voltage (0.1 ms duration) and at 10.0 Hz until a maximum CP developed. Maximal CP responses at each voltage usually occurred within 10–15 s of beginning stimulation. Stimulation was then terminated and recovery occurred within 5–10 s. Threshold and maximum responses in NMT were achieved at lower voltages than those necessary for CP. Accordingly, voltage was increased for full development of the latter and then increased further, reaching levels as near 2 × threshold as possible. In some cases CP declined with increasing voltage. When this occurred, voltage was reduced to maintain a maximal CP. Stimulation of the preganglionic trunk was then repeated at least 4 times with 3–4-min intervals of rest. The elicited CP and NMT responses served as controls for subsequent drug exposure.

Guanethidine sulfate (5.0 mg/kg; Ciba, Summit, NJ) was then injected in bolus via the right external carotid artery and stimulation repeated. Additional guanethidine was given until the pressor response was reduced by at least 50%, generally this occurred at cumulative dose levels of less than 1.5 mg/kg. Pilot studies showed that it was possible to produce reductions in the elicited CP without greatly reducing NMT, MAP or resting CP. This was desirable since depressions of the latter diminished the vasodilator response to injected acetylcholine chloride (10 μg/kg; Sigma, St. Louis, MO) or papaverine chloride (1 mg/kg; Sigma, St. Louis, MO), suggesting that the capacity to evoke a relaxation in the cranial circulation by stimulation of the SCG could also be compromised.

Prostaglandin $F_{2\alpha}$ (2 μg/kg; Sigma, St. Louis, MO) was then injected into the cranial circulation and the stimulations repeated. Prostaglandin $F_{2\alpha}$ increased resting CP but had no influence on mean arterial pressure (MAP) or NMT. Resting CP following $PGF_{2\alpha}$ tended to decline over a period of 20–30 min, such that additional injections of $PGF_{2\alpha}$ (1μg/kg) were given before each stimulation of the SCG. This, with papaverine and acetylcholine controls, insured that resting CP was maintained at comparable starting levels. Atropine sulfate (0.5 mg/kg; Lilly, Indianapolis, IN) was then administered in increasing dose (0.1–0.5 mg/kg) by the carotid route using the loss of response to acetylcholine as end point. The stimulations and recordings were then repeated.

Supramaximal stimulation of the SCG evoked pressor responses in the cranial circulation, and developed tension in the nictitating membrane. One example, typical of the 5 cats is shown in the figure. Data pooled and averaged from these is shown in the table. Resting cranial pressure (RCP; table) increased (ICP) in response to stimulation of the sympathetic preganglionic trunk of the SCG and then decreased (DCP) to less than RCP after the cessation of stimulation. This poststimulation depression of RCP lasted for less than 30 s. Neither the intensity nor length of depression (LCP) were influenced by the length of stimulation of the SCG.

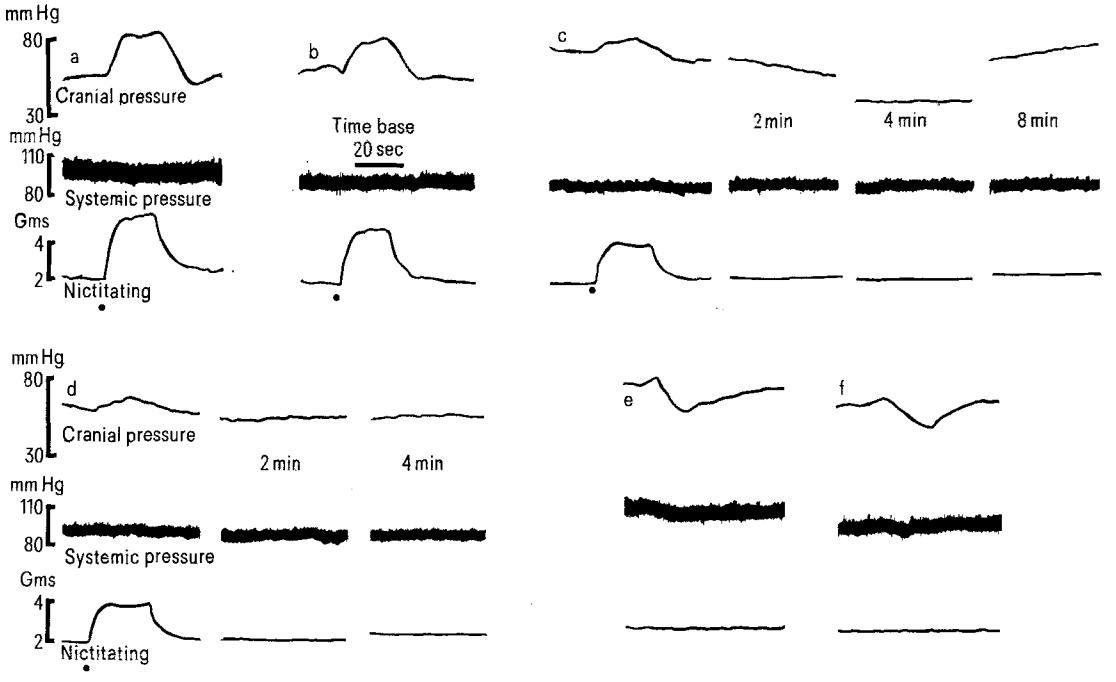
Guanethidine reduced ICP ($p < 0.01$) but not RCP. Mean arterial pressure and NMT were also reduced by guanethidine ($p > 0.02$), but neither DCP or LCP were effected. Prostaglandin $F_{2\alpha}$, while significantly increasing RCP ($p < 0.02$), did not effect ICP or NMT ($p > 0.1$). On the other hand, the poststimulation events were greatly enhanced. Poststimulation, cranial pressure (RCP) continued to decline, reaching 50% of prestimulation RCP after 4 min and then recovering throughout the next 2–4 min.

Atropine had no influence on RCP or ICP but the magnitude of the poststimulation decline in pressure (DCP) was much diminished. Although there was some variability, the period of the depression (LCP) shortened, returning to near that of the predrug controls.

Influence of intracarotid guanethidine, prostaglandin $F_{2\alpha}$ and atropine on mean arterial (MAP) and resting cranial pressure (RCP) before stimulation of superior cervical ganglion. The subsequent columns show the elicited cranial pressure increase (ICP) during stimulation and post-stimulation decrease (DCP) and duration of decrease (LCP) after stimulation. The resting and evoked increase of nictitating membrane tension (NMT) is shown in the last column

Conditions	Sig	MAP (mm Hg)	Sig	RCP (mm Hg)	Sig	ICP (mm Hg)	Sig	DCP (mm Hg)	Sig	LCP (s)	Sig	NMT (g increase)
Control		106.4 ± 5.5		52.6 ± 3.3		29.6 ± 2.7		7.6 ± 0.65		20.1 ± 4.9		1.6 ± 0.2
Guanethidine (mg/kg)	x	85.2 ± 6.1		44.6 ± 4.5		12.4 ± 1.2		6.8 ± 1.0		15.2 ± 5.4		1.1 ± 0.1
Prostaglandin $F_{2\alpha}$ (2 µg/kg)		93.1 ± 4.1	x	68.6 ± 3.5	x	11.6 ± 1.4	x	36.4 ± 3.1	x	240.4 ± 60.0		1.3 ± 0.1
Atropine ! (0.1–0.5 mg/kg)		97.3 ± 5.5		65.5 ± 4.7		11.6 ± 1.2	x	10.0 ± 2.1		44.2 ± 24.3		1.2 ± 0.1

N = 5; x, significant from controls $p < 0.02$; !, following a priming dose of $PGF_{2\alpha}$ (2 µg/kg).



Typical increases in cranial pressure and nictitating membrane tension in response to stimulation of the cervical sympathetic preganglionic trunk of one cat. Stimulation (2 × threshold, 10 Hz, 0.1 ms duration) began in A–D at the 0 and continued for 20 ± 2 s. Controls shown in (A), following guanethidine (1.0 mg/kg) in B, after prostaglandin $F_{2\alpha}$/kg) in C and after atropine (0.1–0.5 mg/kg) in D. Records in E and F show vasodilator controls 3–5 s after close-intracarotid injection of papaverine (1.0 mg/kg) before (E) and after (F) atropine.

Pressure recorded cephalically from one external carotid artery reflects in part the mean perfusion pressures of the contralateral carotid and vertebral artery and the resistances of the diverse vascular bed between. Unlike the in vitro studies, using segments of isolated vascular muscle and transmural stimulations[2–4], the cranial vascular model used in this in vivo study can not differentiate between either vein or arteries or the tissues they subserve, i.e. bone, muscle, skin or neural tissue. However, this and a previous study[1] have shown that mean pressure recorded from this region not only approximates in onset and magnitude respectively the initiation and variations in intensity of stimulation of the sympathetic trunks, but also can detect pharmacologic interventions designed to enhance the influence of at least one mediator – acetylcholine. The development of tension by the nictitating membrane, coincident with stimulation of the SCG, is less sensitive to variations in voltage and frequency than CP, and from this study, more resistant to guanethidine. Nevertheless it is a classical model of a muscle-nerve system, we have continued to use it as an internal control for factors influencing the vasomotor system.

There are some similarities between the results we have obtained in vivo with what others have obtained using transmural stimulation of isolated cranial vessels in vitro. In isolated cranial vessels, subjected to similar pharmacologic treatment, transmural nerve stimulation evoked an initial relaxation followed by a sustained dilation. The latter persisted for 3–5 min beyond the period of stimulation. Only the early effect was blocked by atropine, the nature of the sustained response remained to be established. In our studies there does not appear to be an initial cholinergic vasodilation that is coincident with the period of stimulation of the SCG. Rationally, in the intact cat, it is probably unlikely that a cholinergic vasodilation such as the sympathetic cholinergic 'alarm response'[8,11] could persist for 4–6 min poststimulation. The strong cholinesterase activity in whole blood seems to mitigate against this possibility. It is more likely that a muscarinic receptor triggers the sustained vasodilation, either by releasing a second mediator, or by reversing the effects of $PGF_{2\alpha}$ on vascular smooth muscle.

The neurogenic pathways concerned with these vasodilator responses in the cranial circulation almost certainly are associated

with the SCG; either as a part of the sympathetic preganglionic trunk, or as cholinergic fibers originating near, or in synaptic proximity with the preganglionic sympathetics. On the basis of histochemical studies, others have denied such a possibility, since the large stores of choline acetyltransferase, which appear to be associated with vessels that relax upon transmural stimulation in vitro, are not depleted by section of the sympathetic nerves supplying these regions[2, 10]. Should this be the case, it is conceivable that the trigger for the release of vasodilating transmitter is hemodynamic rather than neurogenic in nature. A local reflex for maintaining central perfusion pressure and regional blood flows during stress has been proposed by others[12] and the present study may provide support for this concept.

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Hydroxyproline concentration in soluble and insoluble material from serum treated with trichloroacetic acid in postpartum mice

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Summary. The hydroxyproline concentration in both the soluble and insoluble material from trichloroacetic acid-treated serum from postpartum mice was determined. The hydroxyproline concentration in the insoluble material increased, but that in the soluble material did not increase during the uterine involuting period.
Key words. Hydroxyproline concentration; serum; trichloroacetic acid; postpartum; mice.

In the mouse, most of the uterine collagen is degraded during the first two postpartum days[1-3]. It is generally thought that collagenase initially degrades collagen fibers to fragments and then other proteolytic enzymes break down these fragments to amino acids[4, 5]. It is not clear to what extent these proteolytic enzymes participate in uterine collagen degradation during the postpartum involution.

Since materials derived from the breakdown of the uterine collagen are removed by the blood stream[6, 7], the occurrence of material containing hydroxyproline (Hyp) in the serum should reflect the collagen-degrading process in the postpartum uterus. We undertook to determine the Hyp concentration in both soluble and insoluble material from serum treated with trichloroacetic acid (TCA), which can fully precipitate large peptides[8], during the first three postpartum days.

Materials and methods. Animals used were female mice of the IVCS strain. They were reared under 12L:12D and given food and water ad libitum. At 8 weeks of age they were mated. All animals were allowed to suckle their pups. The day of parturition was indicated as day 0 postpartum.

Our previous studies[1-3] showed that most of the uterine collagen is degraded during the first two postpartum days. Therefore, blood was collected from day 0 to day 3 postpartum from the femoral artery and vein under ether anesthesia. Only blood samples taken from mice having 8–12 placental scars per pair of uterine horns were analyzed, to avoid any difference due to the number of placental scars[6]; the average number of placental scars in a pair of horns was 9.3 (n = 120). Blood samples taken from 10-week-old diestrous virgin mice served as controls. All blood samples were centrifuged at 3000 rpm for 20 min. The sera of 5 mice were pooled and stored at −20 °C.

One ml 10% (W/V) TCA was added to 1 ml of the serum, which was then centrifuged at 3000 rpm for 20 min. One ml 12 N HCl was added to 1 ml supernatant. The sediment was dried at 100 °C for 24 h and weighed and 2 ml 6 N HCl was added. The samples were hydrolyzed at 130 °C for 3 h. Hyp was determined in these hydrolysates according to Woessner's method[9].

Hydroxyproline concentration in soluble and insoluble material from serum treated with 10% (W/V) trichloroacetic acid during the first three postpartum days

Day after parturition	No of animals[a]	Hydroxyproline concentration Soluble material µg/ml	Insoluble material (dry weight) mg	µg/g
Nulliparous	30	5.1 ± 1.5[b]	55.3 ± 1.1	61.9 ± 8.0
Day 0	30	2.0 ± 0.2	58.9 ± 0.9*	39.4 ± 7.5
Day 1	30	3.0 ± 0.6	66.1 ± 2.6**	83.2 ± 3.8*
Day 2	30	2.1 ± 0.5	71.0 ± 2.2**	92.0 ± 10.3*
Day 3	30	1.5 ± 0.5*	55.3 ± 1.2	50.9 ± 8.5

[a] The serum from 5 mice was pooled. [b] mean ± SE. *$p < 0.05$, **$p < 0.01$ as compared with nulliparous animals (Student's t-test).

Results and discussion. Results are summarized in table 1. The Hyp concentration of the supernatant was the same as that of nulliparous animals from day 0 to day 2 postpartum. The Hyp concentration was decreased on day 3 postpartum. A small increase of the Hyp concentration was found on day 1 postpartum during the first three postpartum days.

The dry weight of the sediment was higher than that of sediment from nulliparous animals from day 0 to day 2 postpartum. The dry weight decreased to the level of that of nulliparous animals on day 3 postpartum. The Hyp concentration of the sediment was not increased on day 0 postpartum but was high on day 1 and day 2 postpartum compared with that of nulliparous animals. The Hyp concentration decreased to the level of that in nulliparous animals on day 3 postpartum.