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Do cholinergic neurons directly innervate nasal blood vessels?

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SUMMARY

The main aim of this work was to provide additional evidence that cholinergic neurons can induce nasal vasodilation by inhibiting norepinephrine release, not by a direct action on nasal blood vessels. We induced a degeneration of the nasal sympathetic neurons in order to observe the effects of electrical stimulation of the cholinergic neurons on nasal blood vessels. Electrical stimulation had no measurable effect. One interpretation of this result is that cholinergic fibers control vasomotor tone by inhibiting the output of the sympathetic neurons, i.e., they have a presynaptic influence. However, it should be stressed that the data were obtained from in vitro experiments, which may not reflect the true in vivo processes. Additional evidence was obtained to demonstrate that electrical stimulation of the in vitro nasal tissue causes contraction by the release of norepinephrine from remnants of sympathetic nerve fibers remaining in the tissue. Also, there is no evidence of transganglionic degeneration in the cervical sympathetic nerve.

INTRODUCTION

The autonomic control of the nasal mucosa has been of clinical and experimental interest for decades. Many of us were taught that the actions of the autonomic nerves in the nasal mucosa were fairly simple. Sympathetic fibers innervated nasal blood vessels. An increase in sympathetic activity caused vasoconstriction, decreased gland secretion and increased nasal patency. Parasympathetic fibers innervated nasal blood vessels and glands. An increase in parasympathetic activity caused vasodilation, gland secretion and a decreased nasal patency. The state of the glands, blood vessels and patency at any moment was the result of these two opposing forces. A clinical condition such as vasomotor rhinitis could thus be ascribed to an overactive parasympathetic system.

However, the discovery and elucidation of the vasoactive intestinal peptide and substance P neural systems in nasal mucosa has changed this simple conception (Änggård, 1981; Lundberg et al., 1981; Lundblad et al., 1983). It has been proposed (Uddman et al., 1981) that neuronal VIP may be the mediator of the atropine resistant vasodilation found by many workers (e.g., Gadlage et al., 1975). Also, other evidence concerning a possible presynaptic, parasympathetic mecha-

nism of nasal blood vessel control has emerged (Jackson, 1982). This latter paper questions the existence of direct cholinergic-blood vessel synapses.

There is good evidence (some of it our own) that parasympathetic nerve stimulation can induce nasal vasodilation (Malm, 1973; Änggård, 1974; Lundblad et al., 1983; Jackson and Rooker 1971; Gadlage et al., 1975; Asakura et al., 1985). The present authors have no argument with this point. However, it is possible that the supposed mechanism for the induction of vasodilation is not completely understood or that there is more than one mechanism available.

An in vitro preparation of dog nasal mucosa has been used to provide the data for the supposed presynaptic mechanism. This preparation was introduced so that the nasal vascular smooth muscle might be examined in an environment less dependent on central nervous control, levels of anaesthesia, changes in blood pressure and blood-borne agents (Jackson 1979, 1980). It is important to note that this is an en masse blood vessel preparation containing arteries, arterioles, capillaries, venous sinusoids, venules and veins. The recorded response is the resultant of the activity of all responding blood vessel muscles. It is currently impossible to know which type of vessel is contributing to the response or if all the vessels are responding in the same way.

The main aim of this study is to provide additional evidence for the hypothesis that parasympathetic, cholinergic neurons do not directly innervate nasal blood vessels in significant numbers. The hypothesis is that an additional mechanism by which cholinergic neurons can induce vasodilation is by inhibiting the sympathetic vasoconstrictor activity by controlling the sympathetic neuron - smooth muscle synaptic activity.

METHODS

The technique and procedure for using the isolated nasal mucosal preparation has been described previously (Jackson, 1979, 1980, 1982; Ichimura and Jackson 1984, 1985). Briefly, one removes the nasal mucosa from the nasal septum of a dog, cuts a piece approximately 5×15 mm and mounts the piece in a muscle bath containing Ringer's solution that is gassed with 95% oxygen and 5% carbon dioxide. Contraction and relaxation of smooth muscle elements in the mucosa caused by drugs or electrical stimulation is detected by a force transducer. Electrical field stimulation of the tissue is accomplished by means of an electronic stimulator (Grass S-4) whose two leads are immersed in the Ringer's solution. When the stimulator is activated, current flows easily through the solution and the tissue and induces smooth muscle contraction. When adrenergic agents such as epinephrine or methoxamine are added to the Ringer's solution, contraction occurs due to direct stimulation of alpha receptors in the blood vessel smooth muscle. When vasodilating agents such as histamine or nitroglycerin are added during electrical stimulation, contraction is inhibited. If the dilating agents

are added to a tissue that has been precontracted by an agent such as epinephrine or methoxamine, the tissue relaxes (Jackson 1980, 1982).

Two methods of unilateral sympathectomy were employed in adult dogs. In two dogs, a 1 cm portion of the sympathetic trunk was excised approximately 1 cm below the superior cervical sympathetic ganglion. The ganglion was left intact. In two dogs, the ganglion itself was isolated and removed. Aseptic technique was employed and each dog was given 1 million units of penicillin immediately after surgery. The dogs were sacrificed 14 days after surgery and the nasal mucosa was removed and tested using the in vitro procedure.

RESULTS

All four dogs showed unilateral ptosis of the eyelid, i.e., Horner's syndrome. All appeared healthy and showed no signs of infection.

Effects of ganglionectomy

Four separate pieces of nasal mucosa were tested from each side of the nose. Electrical stimulation of the unoperated, control side showed the normal contraction spikes usually obtained from this tissue. Electrical stimulation of the tissue from the operated side showed no contractions (Figure 1). The absence of contractions did not depend on the strength of stimulation. 30 V is normally used to elicit a half maximal response. 90 V had no effect on the operated side. Although the usual contraction spikes were absent, the baseline showed a small increase in tension. This may be due to direct electrical stimulation of the smooth muscle itself. Both

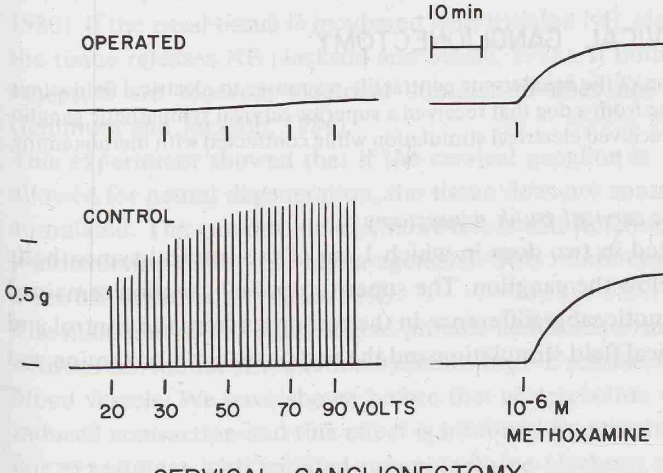
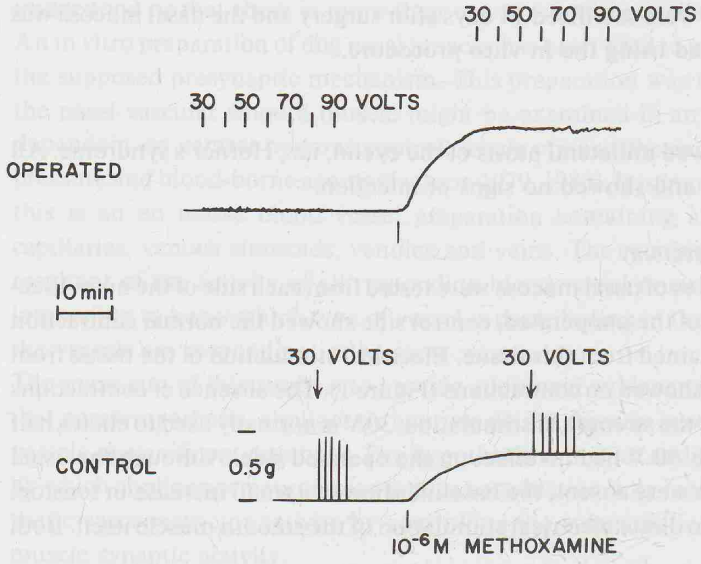


Figure 1 A comparison of the nasal tissue contractile responses to electrical field stimulation and methoxamine from a dog that received a superior cervical sympathetic ganglionectomy.

tissues contracted when treated with 10^{-6} M methoxamine. This implies that the postsynaptic smooth muscle and its alpha receptors were intact and functional. When the tissue was contracted with methoxamine, an alpha agonist, electrical stimulation induced contraction spikes in the control side and had no effect in the operated side. This not only demonstrated that sympathetic innervation was absent from the operated side but it gave no evidence of an operating vasodilating nerve network (Figure 2).



CERVICAL GANGLIONECTOMY

Figure 2 A comparison of the nasal tissue contractile responses to electrical field stimulation and methoxamine from a dog that received a superior cervical sympathetic ganglionectomy. Both tissues received electrical stimulation while contracted with methoxamine.

Effects of sympathetic cervical trunk neurectomy

Nasal tissue was tested in two dogs in which 1 cm of the cervical sympathetic trunk was excised below the ganglion. The superior cervical ganglion remained intact. There was no noticeable difference in the responses from the control and operated side. Electrical field stimulation and the response to methoxamine was the same (Figure 3).

DISCUSSION

One assumption underlying the use of the isolated nasal mucosa preparation is that electrical field stimulation normally causes contraction of the blood vessel smooth muscle by inducing release of norepinephrine (NE) from the sympathetic

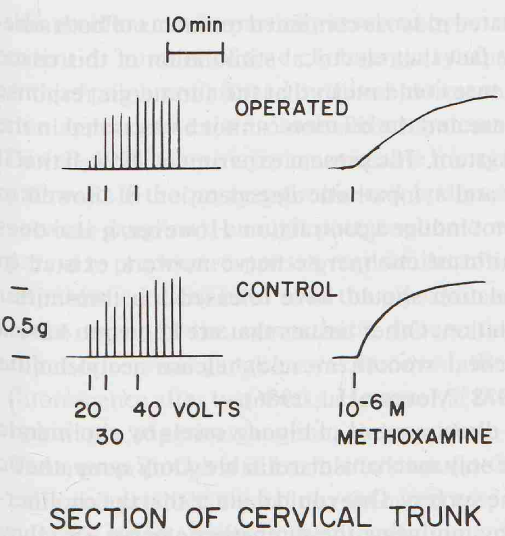


Figure 3 A comparison of the nasal tissue contractile responses to electrical field stimulation from a dog that received a section of the cervical sympathetic trunk below the superior ganglion.

neurons remaining in the mucosa. There is some evidence for this assumption. If one treats the tissue with guanethidine, a drug that interferes with nerve terminal stores of NE, electrical stimulation produces no contractions. This treated tissue will still respond to added alpha agonists such as NE or epinephrine (Jackson, 1980). If the nasal tissue is incubated with tritiated NE, electrical stimulation of the tissue releases NE (Jackson and Steele, 1985). If both alpha-1 and alpha-2 receptors are blocked, electrical stimulation does not cause a contraction (Ichimura and Jackson, 1984).

This experiment showed that if the cervical ganglion is removed and time is allowed for neural degeneration, the tissue does not contract when electrically stimulated. The smooth muscle, however, is still functional. It will respond to methoxamine (and other alpha agonists). This reinforces the assumption that electrical stimulation releases NE.

The main aim of this study was to provide additional evidence that cholinergic neurons can induce vasodilation by inhibiting NE release, not by direct action on blood vessels. We have shown before that acetylcholine will block electrically induced contraction and this effect is inhibited by atropine (Jackson, 1982). In our experiment with tritiated norepinephrine (Jackson and Steele, 1985), we have shown that acetylcholine inhibits the release of norepinephrine and the inhibition is blocked by atropine. Both effects are cholinergic and both are presynaptic.

One could assume that normal isolated mucosa contained remnants of both adrenergic and cholinergic fibers. The fact that electrical stimulation of this tissue routinely causes a contractile response could mean that the adrenergic response overwhelms the cholinergic response and the dilation cannot be recorded in the presence of an intact sympathetic system. The present experiment showed that if the cervical ganglion is removed and sympathetic degeneration is allowed to occur, electrical stimulation does not induce a contraction. However, it also does not induce a relaxation. If a significant cholinergic nerve network existed in contact with blood vessels, stimulation should have released the transmitter (acetylcholine) and caused vasodilation. Other tissues that are known to have a cholinergic innervation (e.g., tracheal smooth muscle) release acetylcholine when field stimulated (Russell, 1978; Moore et al, 1986).

This provides more evidence that direct control of blood vessels by cholinergic neurons is not the preponderant or only mechanism available. Only sympathetic fibers were directly destroyed by the surgery. One could deduce that the cholinergic fibers control vasomotor tone by inhibiting the sympathetic nerve, i.e., they have a presynaptic influence.

Other explanations are possible. Perhaps the in vitro preparation does not demonstrate relaxation easily. Even though we have demonstrated previously that the precontracted tissue will relax when treated with histamine, nitroglycerin, isoproterenol, xylocaine and procaine, we have not been able to show a relaxation with acetylcholine (Jackson, 1982). Perhaps the lack of response has to do with the endothelial relaxing factor (Owen and Bevan, 1985). If this factor is missing, acetylcholine may not induce a response or the response may be greatly reduced. It may be that our handling of the nasal tissue destroyed the factor. We were able to show that acetylcholine inhibited the electrically stimulated response (which would lead to vasodilation) and this effect of acetylcholine was blocked by atropine (Jackson, 1980, 1982).

Another possibility is that direct cholinergic control of vasodilation only occurs in selected types of vessels. Such vessels, e.g., venous sinusoids, could have a large effect on tissue bulk and nasal resistance even though they do not constitute the majority of vessels in the mucosa. Our tissue is usually taken from the nasal septum of the dog although the dorsal wall gives the same responses. The same responses have also been recorded from rabbit nasal tissue. It is possible that the mucosa of the turbinates behaves in a different manner.

The data that we have obtained with the in vitro preparation is not in conflict with in vivo experiments. Our proposal does not deny that parasympathetic vasodilation occurs. It does not deny the existence of a cholinergic synapse directly on nasal blood vessel smooth muscle. It is concerned with other possible mechanisms of cholinergic vasodilation. It was because our in vitro data did not fit the standard scheme that we were forced to look for alternate explanations. Perhaps

this problem can be partially resolved by an ultrastructural demonstration of the extent or number of true cholinergic synapses on nasal blood vessels.
A third point is reinforced by this experiment. Although transneuronal degeneration appears to exist in some other systems (Ghetti et al., 1975; Pinching and Powell, 1971), there is little noticeable transganglionic nerve degeneration in this pathway. If the postganglionic cell bodies are not removed (i.e., the superior cervical ganglion), enough postganglionic neurons persist for the 14 day time period to produce responses very similar to control tissue. We (H-W Wang et al., unpublished data) have used the glyoxylic acid catecholaminergic histofluorescence to study the sympathetic denervation of the nasal mucosa. After the superior cervical ganglion was removed, there was no catecholaminergic histofluorescence after two weeks. However, if the cervical sympathetic trunk was cut below the superior ganglion, there was no visible difference in fluorescence. Thus, even though it is feasible to stimulate the sympathetic trunk preganglionically and produce a good physiological response, one cannot insure removal of neurons by interrupting the preganglionic pathway.

ZUSAMMENFASSUNG

Das Hauptziel dieser Arbeit lag darin, zusätzliche Hinweise dafür zu erbringen, dass cholinerge Neurone eine Gefässerweiterung in der Nasenschleimhaut induzieren, und zwar nicht durch direkten Einfluss auf die Blutgefässe, sondern durch Unterdrückung der Ausschüttung von Norepinephrin. Wir verursachten die Degeneration der sympathischen Innervation der Nase, um die Wirkung elektrischer Reizung von cholinergen Neuronen auf die Blutgefässe der Nase zu beobachten. Doch die Reizung zeitigte keinen messbaren Effekt. Wir interpretieren dieses Resultat dahingelend, dass die cholinergen Fasern die Gefässe durch Inhibition sympathischer Neurone kontrollieren, d.h. die cholinergen Fasern üben einen präsynaptischen Einfluss aus. Wir geben jedoch zubedenken, dass die in vitro durchgeführten Experimente nicht unbedingt die natürlichen Vorgänge in vivo widerspiegeln.
Ein zusätzlicher Hinweis wurde dafür erbracht, dass die in vitro durchgefüphrte Reizung der Nasenschleimhaut eine Kontraktion infolge einer Norepinephrinausschüttung aus überblebseln von sympathischen Nervenfasern in dem Gewebe verursacht. Es fand sich kein Hinweis für eine transganglionäre Degeneration des zervikalen Sympathicustranges.

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