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The inhibition of norepinephrine release in nasal mucosa by acetylcholine

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SUMMARY

Tritiated norepinephrine was incorporated into isolated dog nasal mucosa. Electrical stimulation of this mucosa induced a release of the tritiated norepinephrine. Acetylcholine inhibited this release in a dose-related manner. The inhibition is prevented by atropine. This data provides further evidence for the existence of presynaptic, inhibitory, muscarinic receptors on the sympathetic nerve terminals in nasal blood vessels.

INTRODUCTION

The view of the autonomic innervation of the nasal mucosa used to be simple. Sympathetic nerve fibers were said to innervate the smooth muscle of arteries, veins, sinusoids and vessels controlling blood flow into nasal glands. Stimulation of these neurons resulted in the release of norepinephrine which produces vasoconstriction, decreased gland secretion and a patent nasal airway. Parasympathetic nerve fibers were said to innervate nasal blood vessels and glands. Stimulation resulted in the release of acetylcholine which produced vasodilation and increased gland secretion.

The sympathetic portion of this scheme can be easily corroborated. Nasal sympathetic nerve-muscle transmission is cocaine sensitive, blocked by alpha blocking agents and it responds to exogenous norepinephrine (NE) with vasoconstriction. Experiments with the parasympathetic portion of this scheme produced equivocal results (Malm, 1973; Eccles and Wilson, 1974; Änggård, 1974; Gadlage et al., 1975). Administration of exogenous acetylcholine (ACh) produces small blood vessel dilation responses or none. This could reflect the finding of Änggård and Densert (1974) that "cholinergic" nerve terminals were only 1/10 the number of adrenergic terminals around nasal blood vessels. Efforts to block these responses or the effects of parasympathetic nerve stimulation with atropine gave poor results (Lundberg et al., 1981).

It was stated that these junctions were atropine resistant or not truly cholinergic (Gadlage et al., 1975).

Recently, one of us (Jackson, 1982) has shown that an in vitro preparation of dog nasal blood vessels responds well to both ACh and atropine, in distinction to in

vivo results. Electrically-induced contraction of these vessels is inhibited by ACh in a dose-related manner. This inhibition is blocked by atropine. ACh has no effect on relaxed nasal blood vessel smooth muscle or those vessels previously contracted by NE or methoxamine. Other relaxing agents such as histamine, nitroglycerine and isoproterenol relax such a previously contracted nasal vessel. This implies that there are receptors for histamine, nitroglycerine and isoproterenol on the smooth muscle and there are no receptors for ACh on the smooth muscle. Since electrical stimulation induces the release of NE and ACh affects these contractions, we thought that the ACh might modulate the release of NE from the nerve ending.

The present experiment was designed to measure the effects of ACh on tritiated NE release from electrically stimulated nasal mucosa.

METHODS

The nasal mucosa used in these experiments was obtained from mongrel dogs. The animals were sacrificed with an overdose of sodium pentabarbital supplemented by KCl. The dorsum of the nose was cut with an oscillating bone saw. After removal of the nasal turbinates, the nasal respiratory mucosa was stripped from the nasal septum and dorsal nasal wall. A piece approximately 2×4 cm was obtained from each side of the septum. The tissue was placed in a jar containing bicarbonate-buffered Ringer's solution and gassed for five minutes with 95% O_2 and 5% CO_2.

Just prior to testing, the mucosa was cut into pieces approximately 0.5×1 cm. A piece was incubated for 15 min at 37 degrees C in 2 ml of the buffered Ringer's solution containing 5.3 µl (lµCi/µl) of tritiated NE (Amersham). This solution contained the isotopic NE, 10^{-4}M ascorbic acid (to prevent oxidation) and 10^{-5}M pargyline (to prevent the degradation of NE). The piece of mucosa was rinsed briefly in Ringer's solution to remove the adherent isotope and was then placed in a plastic superfusion chamber 1.8 cm in diameter with a 1 ml fluid capacity. The chamber was equipped with two platinum electrodes attached to a Grass S4 stimulator. The stimulator was controlled by an interval timer (Figure 1). The tissue was superfused with gassed, buffered Ringer's solution containing the pargyline and ascorbic acid at a rate of 1.5 ml/min by means of a perfusion pump. After 2 min of equilibration, the superfusate was collected in 2 min aliquots.

Beginning at 10 min, the stimulator was turned on for a stimulation sequence of 10 sec ON and 20 sec OFF for a period of 2 min. The stimulus was delivered at 70V, 10/sec with a duration of 5 msec. For some experiments, the tissue was stimulated for three, 2 min intervals with recovery periods in between. In other experiments, the tissue was stimulated for one, 2 min interval.

To test the effects of ACh, the drug was added to the incubation mixture and the superfusion fluid in three different concentrations (0.5, 1.0, 5.0 µg/ml). Three

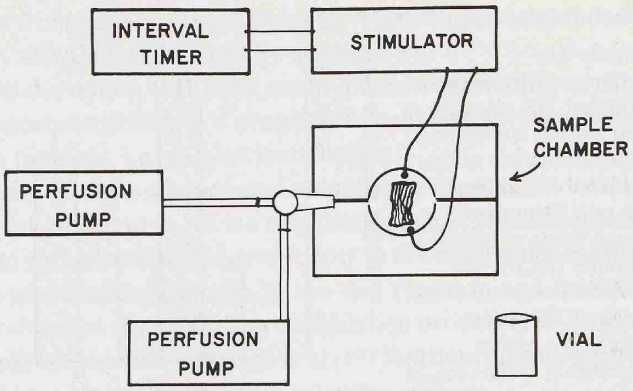

Figure 1.
Diagram of the apparatus used to superfuse and electrically stimulate the mucosa that had been incubated with tritiated NE.

samples of nasal mucosa were tested at each of the three concentrations of ACh and compared to six samples without ACh. To test the effect of atropine, 10 µg/ml was added to the incubation mixture and the superfusion fluid containing 5 µg/ml of ACh in three samples of tissue.

A 0.5 ml aliquot of each collected perfusate was added to a toluene scintillation counting fluid containing 6g/L of PPO, 75 mg/L of POPOP and 10% methyl alcohol and counted for 5 min in a Beckman scintillation detector.

RESULTS

Isolated nasal mucosa incorporated tritiated NE during the 15 min incubation. Superfusion of this tissue removed the isotope from the tissue in an exponential manner. Electrical stimulation significantly increased the amount of tritium recovered in the perfusate. If the stimulation was repeated, the tritium recovered during stimulation decreased (Figure 2).

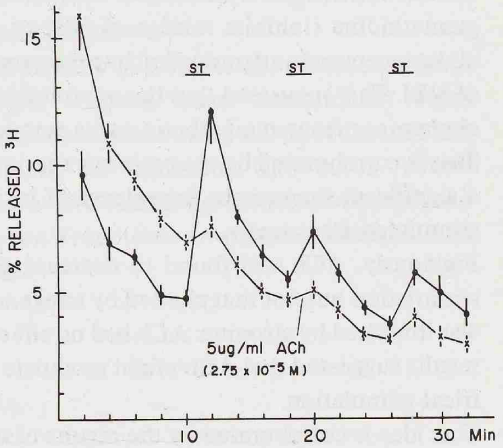

Figure 2.
The percent of tritiated NE recovered versus time of superfusion (+/− S.E.). A comparison of the NE released due to electrical stimulation in the absence and the presence of 5 µg/ml of acetylcholine in the super-fusion fluid. The three bars (at 10, 18 and 26 min) indicate a two min stimulation period (ST). The peaks at 12, 20 and 28 min indicate the response to stimulation.

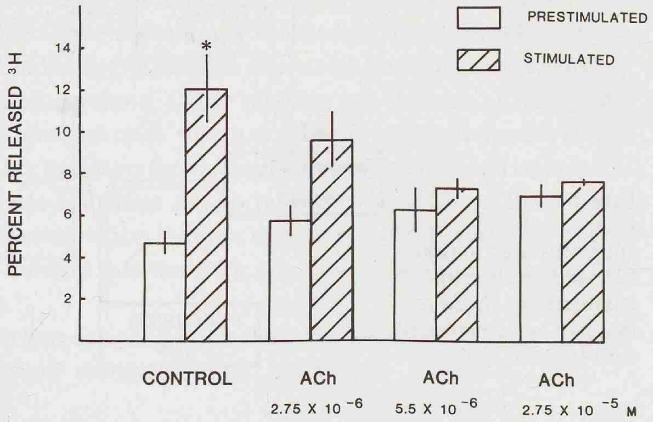

Figure 3.
A comparison of the tritiated NE released at the 10 min collection (prestimulated) to the tritiated NE released at 12 min (stimulated) at three doses of acetylcholine (ACh). The doses correspond to 0.5, 1.0 and 5.0 µg/ml. There is a highly significant difference in the control values ($p < 0.01$).

A portion of the tritiated NE was incorporated into the sympathetic nerve endings and a portion was distributed in the tissue spaces of the mucosa. Contraction of the mucosa induced by exogenous NE did not release tritiated NE.
The amount of tritium recovered during electrical stimulation without ACh is compared to the amount recovered in the presence of ACh in Figure 2. As the dose of ACh increases, the tritium release decreases (Figure 3).
When atropine was added to the incubation mixture with ACh, the tritium release was the same as control values.

DISCUSSION

Previous experiments showed that when the isolated nasal mucosa is stimulated electrically, it contracts. The only tissue component in nasal mucosa capable of contracting in this manner is the smooth muscle of the nasal blood vessels. The contraction elicited by electrical stimulation was blocked by pretreatment with guanethidine (inhibits release of NE) or phentolamine (blocks post-synaptic alpha receptors) and prolonged by pretreatment with cocaine (inhibits the uptake of NE). This suggested that the contraction resulted from the secretion of catecholamines from sympathetic nerve terminals left as remnants in the mucosa. This is corroborated by the present experiments which demonstrate that there is a significant increase in the release of tritiated NE from this tissue when it is stimulated electrically.
Previously, ACh was found to decrease the contraction elicited by electrical stimulation but not that elicited by exogenously applied NE. This effect of ACh was inhibited by atropine. ACh had no effect on the non-stimulated tissue. These results suggested that ACh might modulate the release of NE in response to electrical stimulation.
This idea is corroborated by the results of the present experiment. The stimula-

tion-induced release of tritiated NE was inhibited by ACh in a dose dependent manner and this effect of ACh was blocked by atropine.

This evidence supports the notion that ACh could induce vasodilation in this tissue by inhibiting vasoconstriction via a presynaptic, muscarinic receptor on the sympathetic nerve terminal, i.e. passive vasodilation.

Exogenous ACh does not produce a large nasal vasodilation response in the intact dog. It is certainly not as potent as NE is a constricting agent. However, electrical stimulation of the parasympathetic nerve supply to the nasal mucosa does produce a significant vasodilation response in the dog (Jackson and Rooker, 1971) and the cat (Lundberg et al., 1981). The vasodilation produced by exogenous ACh is largely atropine resistant (Gadlage et al.,1975). These disparities are difficult to reconcile with simple NE and ACh releasing systems.

The demonstration of vasoactive intestinal peptide (VIP) and substance P (SP) containing neurons (Lundberg et al., 1981; Lundblad et al., 1983) in the cat nasal mucosa might provide at least a partial explanation of the disparities.

If VIP or SP is introduced into nasal vessels, they evoke a dilation of nasal vessels that is concentration-dependent. Lundberg et al. have presented evidence that VIP is responsible for the atropine-resistant vasodilation in the nasal mucosa. They showed that ACh and VIP seem to be released simultaneously on parasympathetic nerve stimulation. Their evidence suggests that ACh acts on a presynaptic, inhibitory receptor that modulates the release of VIP.

Evidence such as presented in this paper does not preclude the possibility that parasympathetic neurons release ACh that reacts with postsynaptic, smooth muscle receptors in nasal mucosa and results in vasodilation. The evidence also has little direct bearing on the relative roles of VIP and SP in nasal vasodilation. However, the evidence points to inhibitory, presynaptic control of sympathetic terminals as another factor in this system.

ZUSAMMENFASSUNG

Radioaktives (^{3}H) Norepinephrin wurde aktiv in isolierte Hundenasenschleimhaut aufgenommen. Nach elektrischer Reizung dieser Schleimhaut wurde radioaktives Norepinephrin freigesetzt. Diese Freisetzung wurde in dosenabhängiger Weise von Acetylcholin inhibiert; die Inhibition wird durch Atropin verhindert. Diese Daten sind ein weiterer Hinweis für die Existenz von präsynaptischen, inhibitorischen, muskarinischen Rezeptoren an sympathischen Nervendigungen der Blutgefässe in der Nase.

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