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LACRIMAL SECRETION IN THE CAT

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The lacrimal nerve, a branch of the trigeminal, is mainly a sensory nerve but it also conveys parasympathetic and probably sympathetic fibres to the lacrimal gland (Mutch, 1944). The role of these autonomic fibres in controlling lacrimal secretion is not clear. Stimulation of the lacrimal nerve in dogs was found by Tepliachine (1894) to cause an increase in lacrimal secretion. Botelho (1964) has shown that in cats and rabbits the secretory fibres are derived from the parasympathetic nervous system. In contrast, stimulation of the cervical sympathetic nerves produced variable effects on lacrimal secretion in man and animals (Wolferz, 1871 ; Reich, 1873 ; Schirmer, 1909 ; Duke-Elder, 1932 ; Whitwell, 1961 ; Botelho, 1964).

The experiments described in this paper were undertaken to investigate the effects produced on lacrimal secretion in the cat by stimulation of the lacrimal nerve at different frequencies and to determine whether the secretory fibres were cholinergic.

METHODS

Cats (2 to 4 kg of body weight) were anaesthetized with pentobarbitone sodium (35 mg/kg, intraperitoneally).

Exposure of the lacrimal nerve presented a difficult problem ; the most suitable approach consisted in removing the zygomatic arch after detaching the muscles from it. The nerve could then be located on the lateral aspect of the globe (Fig. 1). A ligature was tied round the lacrimal nerve as far centrally as possible and the nerve was cut proximal to the ligature. Further dissection in preliminary experiments had shown that a small branch of the lacrimal nerve supplied the lacrimal gland and that a larger branch appeared to innervate the superficial structures over the orbit. Since, however, section of the larger branch did not appear to modify the response of the lacrimal gland to nerve stimulation, it was left intact. Thus the lacrimal nerve could be exposed and stimulated at a site where it was readily accessible without dissection or exposure of the lacrimal gland.

Rectangular wave stimuli of supramaximal intensity and 1.5 msec duration were applied to the peripheral end of the cut lacrimal nerve at frequencies ranging from 2 to 30 shocks/sec through bipolar platinum electrodes covered with liquid paraffin (B.P.). The nerve was stimulated for 5 min at a selected frequency and then allowed to rest for 10 min. This sequence was repeated for each of the frequencies used.

The effects of nerve stimulation on lacrimal secretion were compared by a method similar to that described by Schirmer (1909). This consisted of placing into the superior fornix a suitably bent strip of Whatman No. 1 filter paper, 5 mm wide and 5 cm in length. To reduce loss by evaporation, the paper was enclosed in a plastic sheath. The strip was left *in situ* from the beginning of stimulation until immediately before the next stimulation period (a total of 15 min). The time interval

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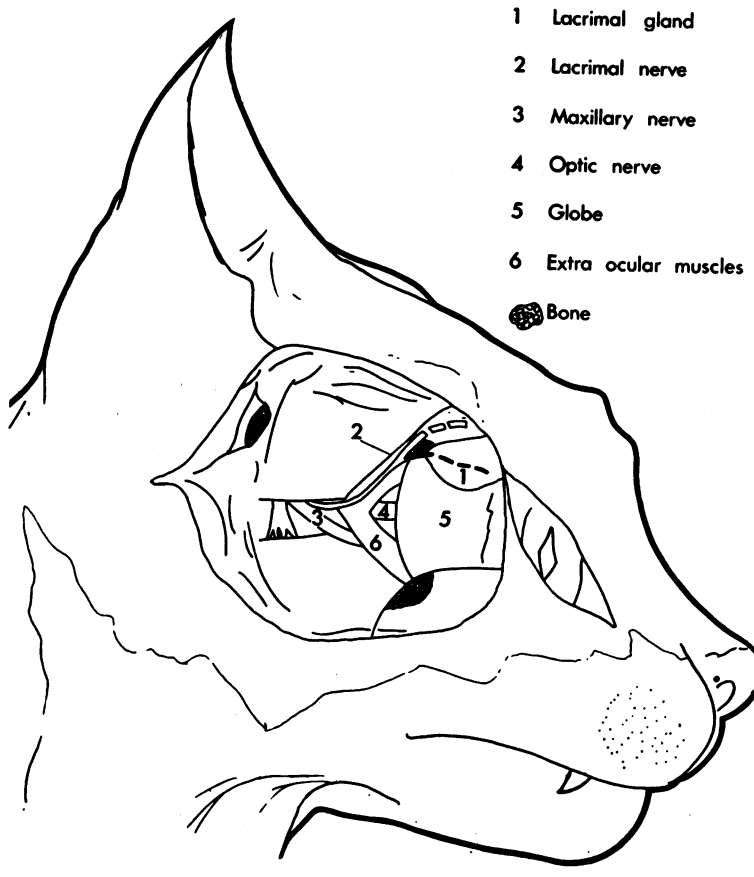

Fig. 1. Dissection of the right orbit showing the lacrimal nerve innervating the lacrimal gland.

between the application of the stimulus and the appearance of a secretion on the filter paper was noted and the amount of secretion expressed as the extent of flow along the paper (mm/15 min); the leading edge was always clearly defined and allowed easy measurement. Control secretions were collected over a period of 15 min without stimulation. In some experiments, a strip of filter paper was also placed in the contralateral superior fornix to determine whether there was any evidence of cross-innervation.

An estimate of the volume of lacrimal secretion was obtained by applying known volumes of 0.9% saline from an Agla micrometer syringe to filter paper strips in plastic sheaths and measuring the amount of wetting after flow ceased, the time for which was usually 2 min.

RESULTS

The effects of lacrimal nerve stimulation on lacrimal secretion were investigated at frequencies ranging from 2 to 30 shocks/sec in six cats. At each frequency there was a delay of up to 2 min before the appearance of a clear watery secretion. The secretion continued to flow along the paper for approximately 1 min after the stimulus had been discontinued. The minimum effective frequency was 5 shocks/sec in five experiments; in one experiment it was 2 shocks/sec.

Figure 2 illustrates the effect of increasing nerve stimulus frequency on secretion. In this experiment the flow was maximum (23 mm/15 min) at 15 shocks/sec ; in the other five experiments maximum flow (range 16 to 27 mm/15 min) occurred at frequencies ranging from 10 to 20 shocks/sec. The control secretions recorded in each experiment immediately before stimulation ranged from 3 to 7 mm/15 min.

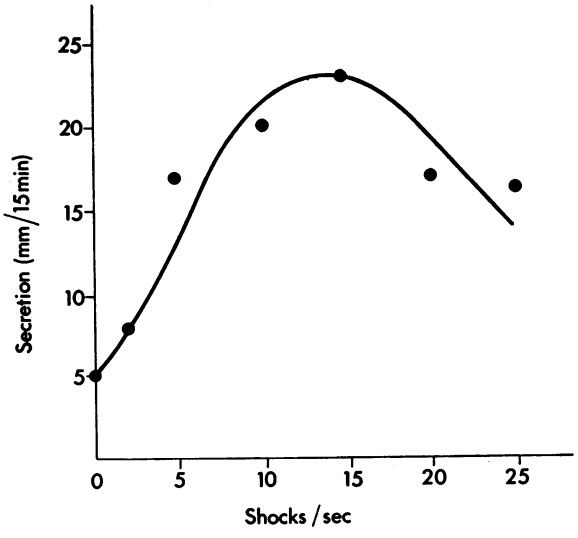

Fig. 2. Cat 2.7 kg. Lacrimal secretion produced by stimulation of the lacrimal nerve at different frequencies.

An estimate of the volume of lacrimal secretion was obtained by applying known volumes of 0.9% saline to filter paper strips and measuring the amount of wetting after flow ceased. Volumes of saline ranging from 2.5 to 15 μl. flowed to the extent of from 1.8 to 2.0 mm/μl., volume and flow being proportional up to 30 mm. Thus the maximum flow of lacrimal secretion observed would probably correspond to a volume ranging from 8 to 14 μl/15 min and the control secretion to a volume of between 1.5 and 3.5 μl./15 min.

In four cats in which a filter paper strip was placed in the superior fornix of the unstimulated side, there was no increase in secretion during lacrimal nerve stimulation of the opposite side, the values remaining within the range of 3 to 6 mm/15 min.

The secretion produced by lacrimal nerve stimulation was clearly derived from the lacrimal gland because it could not be obtained after acute removal of the gland. Thus in two experiments the secretions produced at a frequency of 10 shocks/sec were 10 and 11 mm/15 min before and 3 and 6 mm/15 min after removal of the gland. The control secretions before and after removal ranged from 4 to 6 mm and 3 to 6 mm/15 min respectively.

Intravenous injection of an anticholinesterase compound increased the secretion produced by stimulation of the lacrimal nerve in two cats. For example in Fig. 3 in which the secretion was measured before and 45 min after the administration of 60 μg/kg of physostigmine sulphate, the flow was increased by 86, 47 and 80% respectively at rates

of stimulation of 5, 10 and 15 shocks/sec. There was an increase of 60% in the control secretion after this dose of physostigmine. In another cat a larger dose of physostigmine (375 μg/kg) produced a threefold increase in the secretion at these frequencies compared with the values recorded before the injection.

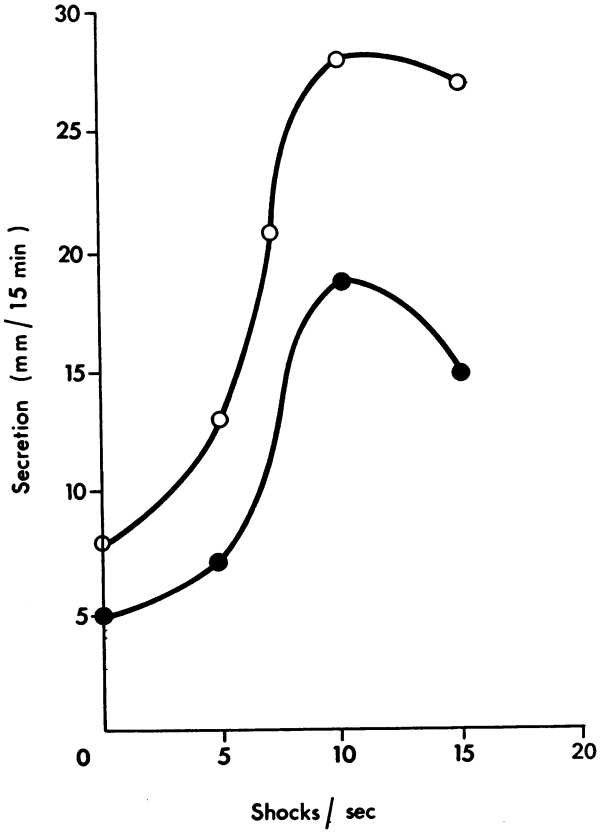

Fig. 3. Cat 4.2 kg. Lacrimal secretion produced by stimulating the lacrimal nerve at different frequencies before (●——●) and 45 min after (○——○) an intravenous injection of 60 μg/kg of physostigmine.

Atropine sulphate administered intravenously in a different dose to each of three cats decreased the secretion produced by nerve stimulation 15 min after the injection. Figure 4 shows that in one cat a dose of 5 μg/kg of atropine abolished the response to stimulation at 5 shocks/sec and reduced the secretion by approximately 50% at frequencies of 10, 15 and 20 shocks/sec. Figure 4 also shows that when the same dose of atropine was injected 90 min after the first the response to nerve stimulation 15 min later was reduced at each frequency to values similar to the control secretion. This dose of atropine did not appear to produce any change in pupillary size.

The injection of 50 μg/kg of atropine in one cat and 250 μg/kg in another promptly reduced the secretion at each of the frequencies of 5, 10, 15 and 20 shocks/sec to values ranging from 6 to 7 and 4 to 6 mm/15 min respectively. These values were similar to the control secretions obtained before and after atropine.

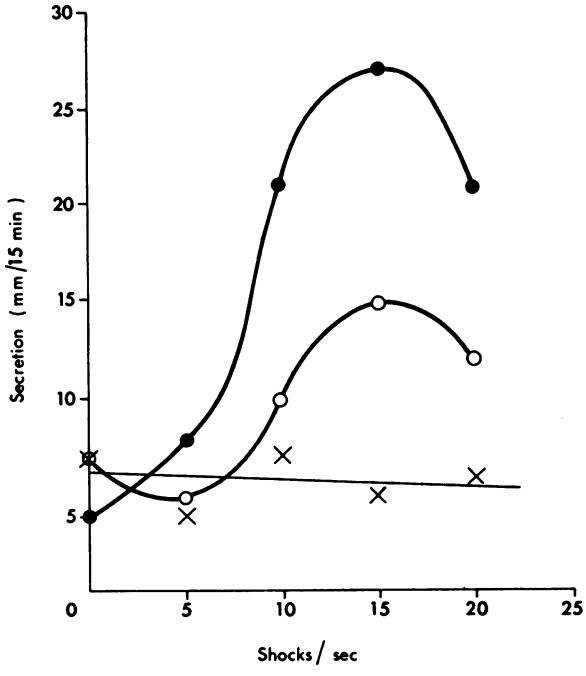

Fig. 4. Cat 2.9 kg. Lacrimal secretion in response to stimulation of the lacrimal nerve at different frequencies before (●——●), 15 min after an intravenous injection of 5 μg/kg of atropine sulphate (O——O), and 15 min after a second similar dose of atropine (×——×) administered 90 min after the first dose.

DISCUSSION

Our attempts to collect lacrimal secretion by cannulating a duct (Botelho, 1964) were unsuccessful because no duct of macroscopic size could be found and histological studies showed only the presence of numerous small ducts. Collection by capillary tube (Emmelin & Strömblad, 1956) was unsatisfactory because it necessitated placing the animal on its back, a posture which rendered nerve stimulation difficult. The secretion was collected therefore from the superior fornix by the method of Schirmer (1909).

Expressing lacrimal secretion as flow along filter paper strips (mm/15 min) is open to the criticism that the amount of secretion absorbed in a given time may not be proportional to the extent of the flow. Experiments showed that volumes of 0.9% saline ranging from 2.5 to 15 μl. flowed to the extent of from 1.8 and 2.0 mm/μl—that is, volume and flow were proportional up to 30 mm. The possibility cannot be excluded, however, that the extent of flow may be modified by any substances contained in it.

A major objection to collecting secretion from the superior fornix is that it consists of the secretion of other glands draining into the conjunctival sac. This applies to the control secretion but not to lacrimal secretion. Indeed it is unlikely that the lacrimal gland contributes to the control secretion because this secretion was not affected by acute removal of the lacrimal gland, nor by doses of atropine which abolished the response to lacrimal nerve stimulation. The glands from which the control secretion was derived did

not appear to be under the influence of the lacrimal nerve because no increase in control secretion was produced by stimulation of this nerve after removal of the gland.

The control secretions obtained immediately before lacrimal nerve stimulation ranged from 3 to 7 mm/15 min. The only other observations on control secretion in a cat were made by Whitwell (1961) who reported flows of 2 and 6 mm after 1 and 4 min respectively. No comment was expressed as to whether these observations were the only ones made.

The secretion produced by lacrimal nerve stimulation was clearly derived from the lacrimal gland because it could not be obtained after acute removal of the gland.

Lacrimal secretion was dependent on the rate at which the lacrimal nerve was stimulated. The minimum effective frequency was between 2 and 5 shocks/sec and maximum flow occurred at frequencies ranging from 10 to 20 shocks/sec. The absence of any increase in secretion from the unstimulated side during stimulation of the contralateral lacrimal nerve indicated the absence of cross-innervation. During the present study no direct comparison was made with other glands but according to Burgen & Emmelin (1961) the maximum rate of secretion of the cat submaxillary gland occurred when the chorda tympani was stimulated at 10 shocks/sec.

There was a delay of up to 2 min following lacrimal nerve stimulation before secretion appeared and secretion continued up to 1 min after stimulation ceased. These delays may depend on the rate at which the filter paper absorbed the secretion. They could probably be more accurately determined by collecting the secretion directly from a duct. Using this method and a 10 sec period of stimulation, Botelho (1964) reported a latency of 2.5 sec with maximum flow at 5 sec and a decrease in secretion during the remaining time the stimulus was applied.

The maximum flow of lacrimal secretion in the present experiments was from 16 to 27 mm/15 min. Expressed as 0.9% saline this would probably correspond to a volume ranging from 8 to 14 μl/15 min. This volume is less than that reported by Botelho (1964) who obtained a flow of 50 μl/sec by cannulation of a duct but no details of the frequency of stimulation were given. The discrepancy between the results of Botelho and those obtained in the present experiments may be due to some of the secretion escaping absorption by the filter paper and draining along the nasolacrimal duct. No precautions had been taken to prevent this occurring.

Physostigmine increased the control secretion. This was probably due to an action on the neuroeffecter junction within the lacrimal gland because the control secretion did not appear to be under the influence of the lacrimal nerve.

The secretion produced by nerve stimulation was also increased by physostigmine suggesting that the lacrimal gland was innervated by cholinergic nerves. This was supported by the finding that atropine inhibited the secretion produced by nerve stimulation.

According to Mutch (1944) and Botelho (1964), the parasympathetic fibres to the lacrimal gland relay in the sphenopalatine ganglion, which suggests that the secretory fibres stimulated in the present experiments are postganglionic, but this requires further investigation.

It is concluded from the studies that, in the cat, the secretory fibres conveyed by the lacrimal nerve to the lacrimal gland are cholinergic.

SUMMARY

1. Electrical stimulation of the lacrimal nerve in the anaesthetized cat produced a secretion from the lacrimal gland which was dependent on the stimulus frequency. The minimum effective frequency was between 2 and 5 shocks/sec and maximum flow occurred at frequencies ranging from 10 to 20 shocks/sec.

2. There was no evidence of cross-innervation to the contralateral lacrimal gland.

3. The lacrimal gland did not appear to contribute to the resting secretion.

4. The secretion produced by lacrimal nerve stimulation was increased by phyostigmine and decreased by atropine. These findings suggest that the secretory fibres conveyed by the lacrimal nerve to the lacrimal gland are cholinergic.

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