*Brit. J. Pharmacol.* (1956), **11**, 147.

# PHARMACOLOGICAL STUDIES ON THE INFERIOR EYELID OF THE ANAESTHETIZED RAT

BY

S. B. GERTNER*

*From the National Institute for Medical Research, Mill Hill, London, N.W.7*

(RECEIVED OCTOBER 14, 1955)

In the present experiments it is shown that the contraction of the inferior eyelid of the anaesthetized rat provides a sensitive method for studying the effects of drugs acting on smooth muscle and sympathetic ganglia. This system has not hitherto been used in the rat, but has been studied in the cat and dog by Isola and Bacq (1946).

## METHODS

Albino rats, approximately 200 g. in weight, were anaesthetized with pentobarbitone sodium (60 mg./kg.) intraperitoneally and fixed in the supine position to a heated metal platform which immobilized the head.

A horseshoe clamp, tightened from below and pressing against the upper canines, held the head down; lateral movement was prevented by an adjustable upright support on each side of the neck and pressing against the skull. The arms and legs of the animal were fixed by string to clamps.

A cannula was inserted into the trachea, and the cervical sympathetic trunk was dissected out under a dissecting microscope, tied peripherally, and cut. The nerve was kept immersed in warm paraffin. Stimulation was by Ag-AgCl electrodes through an RF isolation unit which allowed the animal to be completely dissociated electrically from the stimulating apparatus. It was essential to use an isolation unit; otherwise the effects of cervical sympathetic stimulation were often erratic and uncontrollable. Square wave stimulation was employed throughout at a pulse width of 250 $\mu$sec.

To record the eyelid movements a surgical silk thread was tied through the lower eyelid and fixed by plasticine to a mirror, suspended by a fine, taut wire. A slit of light was focused on the mirror and the leading edge of the reflected light beam allowed to fall on a photocell.

When the eyelid retracted, the beam of light was swept over a greater surface of the photocell so that a proportional movement of the lid was changed to a proportional amount of light illuminating the photocell. The output of the photocell was fed into a D.C. amplifier (Evershed & Vignoles, Ltd., London) matched to an ink writing recorder type PA 10M/A made by the same company. The amplification factor of the amplifier was kept constant throughout the experiments.

The cervical sympathetic trunk was stimulated with supramaximal voltage for periods of 5 sec. All drugs were given intravenously. For this purpose a small glass cannula, connected by a short length of rubber tubing to a mounted 5 ml. syringe, was inserted into the femoral vein. The drugs were injected through the rubber tubing by a fine needle (30 gauge) in a volume of 0.1–0.2 ml. and flushed into the vein with 0.2 saline from the syringe.

## RESULTS

*Stimulation of the cervical sympathetic* for 5 sec. caused an immediate contraction of the inferior eyelid, the degree of contraction depending on the frequency of stimulation. In the experiment of Fig. 1, maximal contraction was observed with a frequency of 32 c/s. In most other experiments, maximal contraction was obtained with 25 c/s.

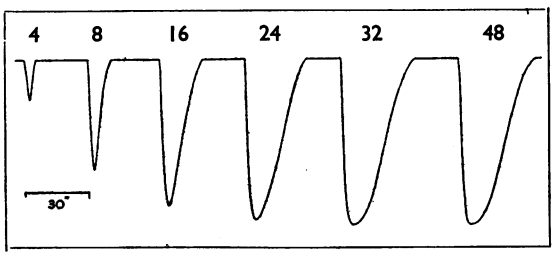


FIG. 1.—Rat, 205 g. Contractions of the inferior eyelid to varying frequencies of stimulation of the cervical sympathetic. Supramaximal voltage; pulse width 250 $\mu$sec., duration of stimulus 5 sec.

*Adrenaline and noradrenaline* produced a contraction of the inferior eyelid. Weight for weight adrenaline was about twice as active as noradrenaline. This is shown in the experiment of Fig. 2. For most animals the threshold dose for adrenaline was about 0.1 $\mu$g. (0.5 $\mu$g./kg.) and for noradrenaline 0.25 $\mu$g. (1.25 $\mu$g./kg.) computed as the base. When the doses were increased, height and duration of contraction increased concomitantly.

* During the tenure of a Fellowship in the Medical Sciences administered by the National Research Council for the Rockefeller Foundation.

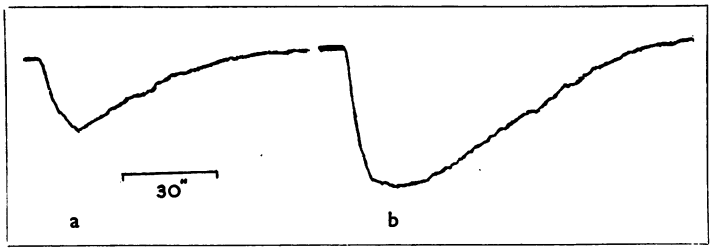


FIG. 2.—Rat, 195 g. Contractions of the inferior eyelid to intravenous administration of 0·5 μg. noradrenaline at a and to 0·5 μg. adrenaline at b computed as the base.

*Acetylcholine*, in doses of 10 μg. (50 μg./kg.) or more, caused a contraction of the inferior eyelid. This is shown in Fig. 3 which, in addition, illustrates the difference in response to a sequence of injections of the same dose of ACh. The contraction to a second injection of ACh was always greatly reduced, yet with subsequent administrations of the drug no further reduction in the response was usually seen.

*Cocaine*, in doses of 2–3 mg./kg., had no action of its own on the inferior eyelid, but enhanced both the responses to cervical sympathetic stimulation and to injections of adrenaline and noradrenaline. The great potentiation of the response to cervical sympathetic stimulation by cocaine is illustrated in Fig. 4. After cocaine, the responses to adrenaline and to noradrenaline became greater and persisted

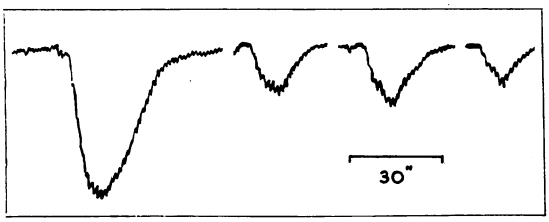


FIG. 3.—Rat, 205 g. Contractions of the inferior eyelid to four intravenous injections of 20 μg. acetylcholine chloride. One min. interval between each tracing.

longer, and a previously subthreshold dose became effective, so that it was often possible to obtain contractions of the inferior eyelid with 0.02 μg. (0.1 μg./kg.) adrenaline and 0.05 μg. (0.25 μg./kg.) noradrenaline. The potentiating effect of cocaine lasted for about 1 hr. It was not possible to detect whether there was a difference between the potentiation of adrenaline and noradrenaline by cocaine.

*Phentolamine* (Rogitine), when injected in doses of 1 mg. (5 mg./kg.), rendered the lower eyelid insensitive to stimulation of the cervical sympathetic and to adrenaline and noradrenaline. The effect was immediate and lasted for about ½ hr.

*Tetraethylammonium chloride* (TEA), *hexamethonium chloride* (C6), and *Ro* 2–2581 [(+)-3,4(1′,3′-dibenzyl-2′-keto-imidazolido)-1,2-trimethylene thiophanium chloride], a short-acting thiophanium ganglion-blocking agent, abolished the effect of sympathetic stimulation on the eyelid. Higher doses of all three, however, were required to produce this effect than in corresponding experiments on the nictitating membrane of the cat. A typical result with Ro 2–2581 is shown in Fig. 5; the injection of 2 mg./kg. greatly reduced, but did not abolish, the effect of cervical sympathetic stimulation.

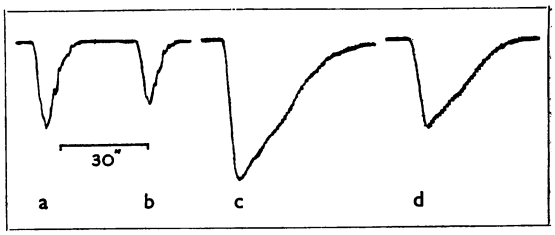


FIG. 4.—Rat, 200 g. Contractions of the rat's inferior eyelid to 5 sec. periods of stimulation of the cervical sympathetic. Potentiation of the response by cocaine. Stimulation at 4 c/s at a and c, and at 2 c/s at b and d; 250 μsec. pulse width. Five min. before c, intravenous injection of 0·4 mg. cocaine hydrochloride.

The action was over in 3 min. In the cat, 2 mg./kg. would have produced complete block lasting for over 15 min. (Randall, Peterson and Lehmann, 1949). In a 200 g. rat complete block occurred only after 5 mg./kg. and never lasted longer than 5 min. TEA produced complete block in doses of the order of 25 mg./kg. and hexamethonium in doses of 5 mg./kg. The block produced by TEA was over within a few minutes; that produced by hexamethonium lasted for about 15 min.

*Eserine* (0.5 mg./kg.) had no action on the lower eyelid and did not affect the response to sympathetic

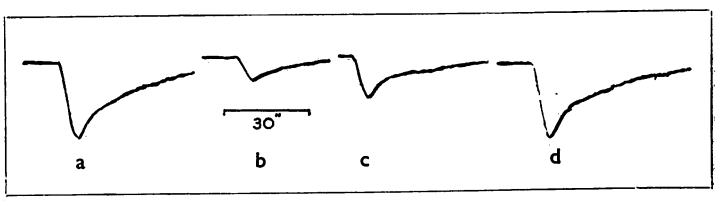


FIG. 5.—Rat, 210 g. Contractions of the inferior eyelid to 5 sec. periods of stimulation (at 4 c/s, 250 μsec.) of the cervical sympathetic before (a) and 1 min. (b), 2 min. (c) and 3 min. (d) after an intravenous injection of 0·4 mg. of Ro 2–2581.

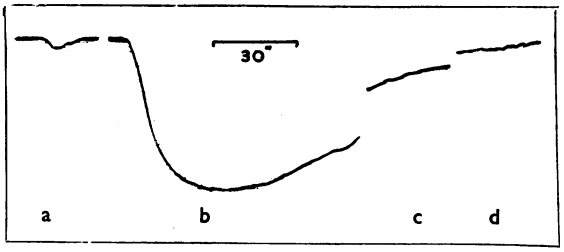


FIG. 6.—Rat, 200 g. Contractions of the inferior eyelid to intravenous injections of 10 μg. acetylcholine chloride, before (a) and after (b) an intravenous injection of 0·1 mg. eserine sulphate. Between (b) and (c), and (c) and (d), one minute intervals.

stimulation, but greatly enhanced the response to ACh (Fig. 6).

*Atropine*, in small doses (0.5 mg./kg.), rendered the eyelid muscle insensitive to ACh, but did not affect its response to sympathetic stimulation, adrenaline, or noradrenaline. Larger doses of atropine (2 mg./kg.) caused relaxation of the eyelid. This effect was small, but occurred regularly. It is illustrated in Fig. 7 by the fact that the record at C starts at a slightly higher point in the tracing than at A and B. The figure further illustrates that, in larger doses, atropine reduced, to a slight extent, the responses both to adrenaline and to cervical sympathetic stimulation. The response to noradrenaline was equally reduced under these conditions. Usually, the effect of atropine lasted for less than ¾ hr.

(+)-*Tubocurarine chloride* in doses which produce arrest of respiration had no action on the eyelid, nor did it affect the response to adrenaline or noradrenaline; but it did cause a slight diminution of the response to stimulation of the cervical sympathetic. This reduction is most likely due to the ganglion-blocking action of curare.

## DISCUSSION

The results suggest that the sympathetic innervation of the smooth muscles of the eyelid of the rat consists solely of adrenergic fibres, and that these muscles receive no cholinergic, postganglionic, sympathetic fibres. This conclusion is based on the findings that cocaine potentiates and phentolamine blocks the effect of sympathetic stimulation to the eyelid; and that neither eserine, nor small doses of atropine which abolish the effect of injected acetylcholine, influence the response to sympathetic stimulation. The ability of atropine in large doses to depress the response cannot be taken as evidence for a cholinergic nerve supply, since it is known (Kuroda, 1924; Schilf, 1927; Feldberg, 1931; Gaddum, 1936) that large doses of atropine have an unspecific depressant effect on smooth muscles.

The finding that the rat eyelid responds to relatively small doses of adrenaline or noradrenaline, particularly after cocaine, and discriminates well between different doses, renders it a suitable preparation for the assay of small amounts of adrenaline or noradrenaline and probably of other sympathomimetic amines as well. The amounts of adrenaline which could be assayed with this method were of the order of 0.02–0.1 μg. The preparation should also prove useful when testing ganglion-blocking agents; because of the smallness of the animal—usually 200 g.—it would be possible to assay such agents when available only in small amounts.

## SUMMARY

1. The contractions of the inferior eyelid of the anaesthetized rat have been recorded. Contractions were produced by stimulation of the cervical sympathetic and by intravenous injection of adren-

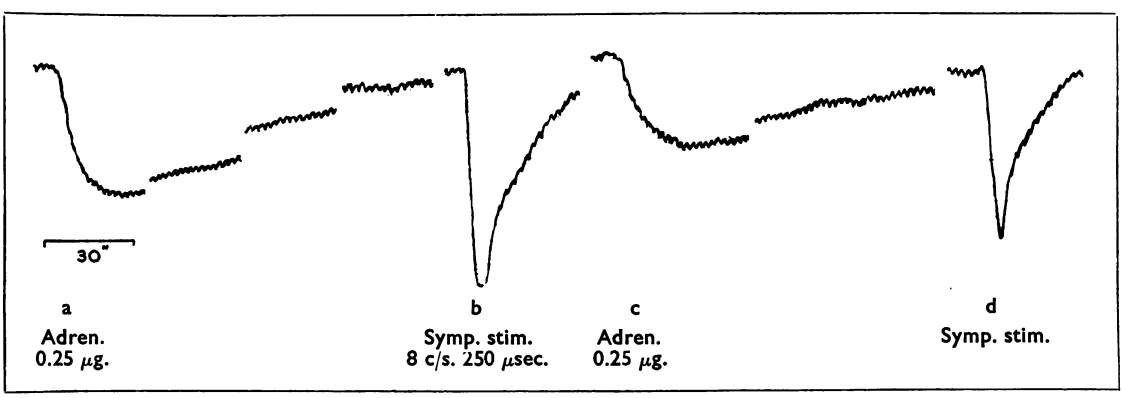


FIG. 7.—Rat, 195 g. Contractions of the inferior eyelid to intravenous injections of 0·25 μg. adrenaline, computed as base (at a and c); and to 5 sec. periods of stimulation of the cervical sympathetic (at b and d) at 8 c/s, 250 μsec. pulse width. Intravenous injection of 0·4 mg. atropine sulphate immediately after b and 5 min. before c.

aline, noradrenaline, and acetylcholine. The contractions to sympathetic stimulation were enhanced by cocaine and abolished by phentolamine, hexamethonium, tetraethylammonium and Ro 2–2581.

2. This preparation is useful for assaying sympathomimetic amines, their antagonists, and ganglion blocking agents—particularly when only a small supply of material is available.

I am indebted to Dr. W. Feldberg for his continued interest during this investigation, to Dr. Laurence Malcolm for help and advice in setting up the experimental arrangement, and to Dr. Elmer Sevringhaus, of Hoffman La Roche, for a generous supply of Ro 2–2581.

## References

Feldberg, W. (1931). *Rev. Pharmacol. Therap. exp.*, **1**, 312.

Gaddum, J. H. (1936). *Gefässerweiternde Stoffe der Gewebe*. Leipzig: Thieme.

Isola, W., and Bacq, Z. M. (1946). *Arch. int. Physiol.*, **54**, 30.

Kuroda, S. (1924). *Z. exp. Med.*, **39**, 341.

Randall, L. O., Peterson, W. G., and Lehmann, G. (1949). *J. Pharmacol.*, **97**, 48.

Schilf, E. (1927). *Autonomes Nervensystem*. Leipzig: Thieme.