# EFFECT OF TEMPERATURE ON THE RESPONSES OF FROG OESOPHAGEAL CILIARY EPITHELIUM TO ADRENALINE, NORADRENALINE, AND ISOPRENALINE

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**Summary :** Isolated mucus membrane strips with attached ciliary epithelium but devoid of muscle were prepared from the oesophagus of frogs. The effects of catecholamines, tolazoline and propranolol at 20-22° and 37° C confirmed the presence of alpha inhibitory and beta excitatory adrenergic receptors in the ciliary epithelium.

The effects of adrenaline and isoprenaline on these receptors were both concentration and temperature dependent. Noradrenaline acted mainly on alpha receptors and depressed ciliary movements at all concentrations and temperatures used. The alpha receptors retained their responsiveness at 10—12° C while the beta receptors became inactive.

**Key words :** frog's ciliary epithelium catecholamines effect of temperature

## INTRODUCTION

Though the action of parasympathomimetic drugs like acetylcholine and physostigmine on the ciliary epithelium of frog's oesophagus, trachea and gillplates of mytilus edulis has been extensively studied by Burn (2, 3, 5), only preliminary work was done with catecholamines (3).

An earlier study undertaken in our laboratory suggested the presence of alpha-inhibitory and beta-excitatory adrenergic receptors in the frog's ciliary epithelium (6). It was observed that noradrenaline was depressant throughout the year with the maximum action seen in winter whereas adrenaline and isoprenaline were stimulant in summer but elicited either depression or initial stimulation followed by depression in the winter in the same concentrations. In this paper we report the effect of temperature on the responses to catecholamines of the ciliary epithelium of frog's oesophagus.

## MATERIALS AND METHODS

The muscle coat of oesophagus of pithed frogs was stripped so that a semitransparent or transluscent strip of ciliated mucus membrane was isolated. A 12 mm long piece was placed in a muscle chamber having a glass cover with a slit in the midline. The strip lay free in the muscle chamber and was neither loose nor had any undue stretch. Through the slit in the glass lid, poppy seeds of uniform size were dropped and the time in sec was recorded till they travelled the distance of 12 mm. Fresh seeds were used every time and the mean time taken by 10 seeds was determined. Ten such experiments were made with each drug concentration.

Strips were bathed with oxygenated frog Ringer solution or solutions of drugs in variou concentrations for 5 min. The temperature of the solutions was kept at the desired level b the use of a thermostatic organ bath. After the 5 min bath at a particular temperature th solution was drained off till the level just touched the under-surface of the strip and the obse vations made as described above.

The effects of noradrenaline bitartrate, adrenaline hydrochloride, isoprenaline sulphate tolazoline hydrochloride and propranolol hydrochloride were studied at 10-12°, 20-22°, and 37°C The effects of catecholamines were also studied after exposure of the strip to alpha and beta-receptor blockers for 5 min. To exclude the possibility of drugs acting through the remaining submucosal coat or muscle fibres from the under surface of the strip, a series of experiments were devised in which the drug solutions were applied to the under-surface only. This procedure did not modify the normal movements of cilia. But when the drug level was further raised to submerge the upper surface also, the typical drug effects were obtained.

The results are expressed as the mean % change in the rate of ciliary movements as described by Burn (3).

## RESULTS

Table I demonstrates that the effect of drugs varied with the concentration used or the temperature at which the response was recorded.

Noradrenaline depressed the ciliary movements at all temperatures and concentrations. Maximal effect was seen at 10-12° C and at 200 $\mu g$ concentration. Further increase in either the concentration or the temperature produced less depression of the ciliary movements.

Adrenaline and isoprenaline were depressant in all the concentrations used at 10-12° C., but elicited a biphasic effect at 20-22° and 37° C. Lower concentrations i e 10, 100, and 200 $\mu g$ were stimulant to the cilia while the maximum concentration i e 1 mg, was depressant.

50 $\mu g$ of tolazoline did not significantly depress the ciliary movements (-13.8 $\pm$ 0.1 at 10° C.-9.7 $\pm$ 0.1 at 20-22° C and -6.5 $\pm$ 0.1 at 37° C). However, it blocked the noradrenaline, adrenaline and isoprenaline-induced depression of cilia at all temperatures and potentiated the stimulant effects of adrenaline and isoprenaline at 20-22° C and more so at 37° C. Propranolol (50 $\mu g$) was slightly stimulant to the ciliary movements ( +7.6 $\pm$ 0.1 at 10° C, + 12.2 $\pm$ 0.2 at 20-22° C and —13.4 $\pm$ 0.2 at 37°C), yet it effectively blocked the stimulant effect of adrenaline and isoprenaline at 20-22° C and 37° C and potentiated the depressant effects of 1 $mg$ adrenaline at 10-12°, 20-22° and 37° C. However, propranolol failed to antagonise the depressant effects of noradrenaline at 10-12°, 20-22° and 37° C.

Finally as is evident from Table I, with the decrease in temperature from 37° C to 20-22° or 10-12° C, the stimulant effect of the catecholamines diminished and the depressant effect increased progressively.

TABLE I : Effects of catecholamines on the movement of cilia of Frog's oesophagus and their antagonism by Tolazoline and Proptranolol at different temperatures.

| *Drug and concentration* | *Mena % change in rate of ciliary movement ± S.E. (Each value is the mean of 10 observations)* | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | *Tolazoline* | | 50 μg | *Proptranolol* | | 50 μg |
| | 10-12°C | 20-22°C | 37°C | 10-12°C | 20-22°C | 37°C | 10-12°C | 20-22°C | 37°C |
| Noradrenaline | | | | | | | | | |
| 10μg | —36.8±.3 | —32.1±.2 | —23.8±.3 | —2.0±.1 | —11.3±.2 | —8.8±.1 | —32.6±.2* | —34.2±.4* | —28.2±.2 |
| 100 μg | —38.2±.2 | —33.3±.3 | —31.2±.3 | —5.7±.1 | —13.5±.2 | —9.3±.1 | —40.8±.4* | —42.8±.5 | —38.2±.4 |
| 200 μg | —67.9±.8 | —61.3±.5 | —59.3±.3 | —8.5±.2 | —42.5±.4 | —35.2±.3 | —70.3±.9* | —61.7±.8* | —65.3±.7 |
| 1 *mg* | —55.9±.3 | —49.2±.4 | —35.0±.4 | —11.7±.2 | —39.1±.3 | —32.1±.3 | —59.7±.6 | —44.2±.4 | —43.3±.4 |
| Adrenaline | | | | | | | | | |
| 10 μg | —15.3±.3 | +70.4± .5 | +107.8±.8 | +10.6±.2 | + 78.6±.7 | +130.8±.9 | —20.6±.3 | —18.9±.2 | —20.2±.2 |
| 100 μg | —20.1±.1 | +54.0± .4 | +89.±3.8 | +16.8±.2 | + 65.3±.5 | +124.6±.9 | —21.7±.1* | —25.3±.2 | —26.8±.3 |
| 200 μg | —23.7±.2 | +39.3± .3 | +58.2±.7 | +18.7±.2 | + 43.5±.5 | + 70.1±.7 | —23.1±.2* | —20.7±.2 | —16.5±.1 |
| 1 *mg* | —26.1±.3 | —20.7±1.0 | —15.5±.2 | +15.0±.2 | +102.7±.9 | +163.5±1.0 | —30.8±.3 | —32.8±.2 | —27.2±.2 |
| Isoprenaline | | | | | | | | | |
| 10 μg | — 6.3±.1 | +42.1±.4 | +130.3±.8 | +12.8±.2 | + 89.8±.9 | +150.0±.8 | —15.2±.3 | —17.6±.3 | —15.7±.2 |
| 100 μg | —18.2±.2 | +53.3±.3 | +125.6±.3 | +18.7±.2 | +108.4±.9 | +128.4±.8 | —27.8±.2 | —13.7±.2 | —16.5±2. |
| 200 μg | —27.9±.3 | +61.3±.5 | + 86.1±.5 | +28.6±.3 | +122.6±1.0 | +142.1±.9 | —30.7±.4* | —11.8±.2 | —19.1±2. |
| 1 *mg* | —45.9±.3 | —39.2±.4 | — 23.9±.3 | +15.5±.5 | +138.1±1.0 | +120.7±.9 | —45.7±.7* | —18.6±.2 | —16.1±2. |

Mean control rate of ciliary movements at :

10 — 120°C = 40 sec for 12 mm

20 — 22°C = 24 sec for 12 mm

37°C = 18 sec for 12 mm

— = % decrease in control rate

— = % increase in control rate

Probability of all the values except marked was* between $<0.05$ and $>0.01$

## DISCUSSION

The effects of catecholamines and their antagonism by alpha and beta-blocking drugs at 20-22° and 37° C, confirm our previous observation of the presence of alpha-inhibitory and beta-excitatory adrenergic receptors in the epithelial cells of frog's oesophagus. The alpha receptors are excited by noradrenaline and higher concentrations of adrenaline and isoprenaline and blocked by tolazoline. The beta receptors are excited by the lower concentrations of isoprenaline and adrenaline and adrenaline and can be blocked by propranolol (6).

In this study all the three catecholamines produced depression of ciliary movement at 10-12° C. This could be due to exclusive excitation of alpha receptors at this temperature. Both adrenaline and isoprenaline produced depression of cilia in the concentrations which were distinctly stimulant to the ciliary movements at higher temperatures and the depression could be blocked by tolazoline. This suggested a transition from beta receptor activity as the temperature of ciliary epithelium is lowered. This is consistent with the observations of Kunos and Szentivanyi (7) who showed that cooling of the isolated frog heart resulted in a transition from beta to an alpha receptor activity. Buckley *et al* (1) however, demonstrated that this was due to the presence of two distinct pools of adrenergic receptors in isolated frog heart, excitation of which was governed by temperature.

Price *et al.* (8) have also shown that beta-receptors in dog heart lung bypass preparation became ineffective at 15° C., whereas alpha receptors retained their effectiveness. Isoprenaline is known to excite alpha receptors in the presence of a beta receptor blockade. Thus the depressant effect of isoprenaline in lower concentration on ciliary movements could be due to excitation of alpha receptors having been rendered ineffective by the lowering of temperature to 10-12°C.

Further, noradrenaline became more depressant as the temperature of the preparation was lowered while adrenaline and isoprenaline (10 $\mu g$, 100 $\mu g$ and 200 $\mu g$) became less stimulant upto 20-22°C. Further lowering of temperature to 10-12°C resulted in complete depressant effect. This could be due to the activation of both the alpha and beta receptors at 37° and 20-22° C, the effect of alpha receptor activation being masked by that of predominant beta-receptors stimulation, as blockade of these receptors with propranolol resulted in an alpha receptor activity.

The inhibitory effect of adrenaline and isoprenaline at 20-22° and 37° C is concentration dependant. Adrenaline is known to excite beta receptors at concentrations lower than those needed for exciting alpha receptors. It caused stimulation of cilia in lower concentrations (10 $\mu g$, 100 $\mu g$ and 200 $\mu g$) and depression in higher concentration (1 *mg*) (6) when the concentrations were increased from 10 to 100 $\mu g$ or 200 $\mu g$ the stimulant effect progressively diminished till it caused depression in the concentration of 1 *mg*. Thus at lower concentration beta receptor excitation predominates over that of alpha-receptors while increasing the concentra-

tion results in progressively more excitation of alpha receptors which finally masks the effect of beta receptor excitation.

Isoprenaline induced depression instead of stimulation in the higher concentration of 1 *mg*. This could be due to beta-receptor blockade as suggested by Butterworth (4) or due to direct stimulation of alpha-receptor by isoprenaline as tolazoline completely reversed this effect.

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