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REVERSAL OF THE ACTION OF ADRENALINE ON A SINGLE MELANOPHORE IN THE FROG (*RANA TEMPORARIA*)

BY

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Dale (1913) showed that the action of adrenaline on the isolated ferret's uterus was to cause an increase of tone, but if ergotoxine preceded the adrenaline the effect was a decrease of tone. Subsequently, Spaeth and Barbour (1917) found that the melanophores of an isolated fish scale (*Fundulus heteroclitus*), which are normally "contracted" by adrenaline, were "dilated" by adrenaline if they had been previously treated with ergotoxine. Houssay and Ungar (1925), reporting on the action of various drugs (injected into the ventral lymph sac) on the colour of the frog's skin, found that both adrenaline and ergotoxine caused the skin to become lighter in colour, but if adrenaline was given after ergotoxine there was darkening of the skin. Hou (1930) confirmed Spaeth and Barbour's work, and also obtained a similar result using ephedrine in place of adrenaline. Leszczynski (1933) showed that when adrenaline was injected into the amputated legs of a frog the skin became paler, but if ergotamine was injected into the lymph sac before the amputation an adrenaline injection into the amputated legs resulted in the skin becoming darker. Shen (1937) obtained a reversal of the action of adrenaline on the melanophores of the frog's skin with ergotamine, F933, and yohimbine. However, Frommel and Zimmet (1937), using 500 μg. ergotamine and 100 μg. adrenaline injected into the lymph sac of a frog, did not observe any alteration of the action of adrenaline on the skin colour by the previous injection of ergotamine.

Although the melanophores of normal frogs "contract" when exposed to light, pithed or urethanized frogs do so to a much smaller degree (Kobayashi, 1928). Yamaguchi (1929) showed that melanophores are sensitive to temperature changes, and suggested that these responses might obscure the effects of drugs. There are, also, seasonal variations in the response of the frog's blood vessels to adrenaline dosage (Karasek and Poupa, 1937), and this may apply to the sensitivity of melanophores.

It is now generally agreed that the melanophore does not "contract" or "expand," but that the pigment moves; therefore the terms dispersion and concentration are used, rather than dilatation and contraction.

Previous workers did not observe the responses of the same melanophore to both adrenaline and ergotoxine, and therefore the question whether the action of ergo-

toxine was an unmasking of an adrenaline dispersing effect, or an actual reversal of the action of adrenaline, remained unanswered. The present work on the responses of one selected melanophore was undertaken in order to provide an answer to this question.

The effect of ergotoxine on the blood vessels occurs quickly, whereas the response of the melanophore is slow. Doses of ergotoxine large enough to ensure concentration of the melanophore and persistence of action, so that the subsequent effect of adrenaline is reversed, are likely to introduce an error owing to metabolic changes caused by ischaemia (Uyeno, 1922).

METHOD

The method used was to examine the skin of a frog's web microscopically, using a magnification of 480. A single cell was used for all measurements in a given series, and the measurements made were always along the same axis of the cell. In effect, therefore, the variable recorded is the linear dispersion of the pigment in certain processes of the melanophore. By means of an eyepiece scale these distances were recorded in arbitrary lengths. As occasionally the pigment may flow out and back in a few minutes, measurements were made every minute. Sometimes, when the melanophore is in the concentrated stage the cytoplasmic outline of the cell may be clearly seen, and care was taken to measure the span of the pigment and not of the cell boundary.

The melanophores in the apex of the web just adjoining the digits of the foot give the most reproducible results, and throughout these experiments all observations were carried out on cells in this region.

Pithed frogs were used, the body being supported by a cork platform held by two clamps and the web of one foot fixed to a ring of cork cemented to a glass slide. In order to ensure that the same cell was observed throughout the procedure, the glass slide was clipped to the movable stage of the microscope, and when a suitable area of melanophores was found the vernier scale reading was recorded. Further, the area being observed was marked off and the local distribution of blood vessels recorded by a line diagram. By these methods the frog could be removed and later replaced, the same cell being observed in the same orientation.

The experiments were carried out during the four months of July to October, and the room temperature varied between 17 and 20° C. The background colour was the same, and the intensity of illumination was kept approximately constant. All drugs were injected intravenously by means of a cannula in the anterior abdominal vein.

Ergotoxine (35 μg./10 g. frog) is insufficient to give rise to gross impairment of the peripheral circulation but sufficient for the experimental procedure, provided the subsequent adrenaline injection follows within a minute.

The standard dose for concentration of the pigment was 2 μg. adrenaline (frog weight varied between 18 and 26 g.), and it was always found that 8 minutes later the pigment was concentrated. Thereafter, a slow dispersion occurred.

In each experiment the rate of dispersion was first estimated as the time taken (dispersion time), from the moment of injection of adrenaline, for the melanophore to reach an arbitrary size of 20 scale units (about two-thirds full dispersion).

The following procedures were then carried out to ascertain whether there was an alteration of the dispersion time, measured from the initial 2 μg. adrenaline injection to standard size of 20 scale units.

(1) Adrenaline, 2 μg.; 8.5 minutes later adrenaline, 2 μg.

(2) Adrenaline, 2 μg.; 8 minutes later ergotoxine (35 μg./10 g. of frog); 0.5 minute later adrenaline, 2 μg.

(3) Adrenaline, 2 μg.: 8 minutes later ergotoxine (35 μg./10 g. of frog); 0.5 minute later 0.1 c.c. of saline.

(4) Adrenaline, 2 μg.; 8 minutes later ergometrine (120 μg./10 g. of frog, as this dose was found to be the maximum that could be safely given); 0.5 minute later adrenaline, 2 μg.

The dispersion times in all these procedures are recorded in the Table. Each experiment involving all these procedures was carried out in a random order, and 24 experiments were performed.

Ergometrine has no or little adrenolytic action, and it was used to ascertain whether it would fail, as in mammalian work (except in very high dosage), to reverse the action of adrenaline.

It was found that if the melanophores were submitted to repeated injections of adrenaline, ergotoxine, or ergometrine, the melanophore became concentrated and unresponsive to all drugs. By keeping the frog at 4° C. for a few hours after each procedure, this difficulty was overcome. It was therefore convenient to carry out each procedure in a session, either morning or afternoon, so that a complete experiment lasted 2–3 days. Fortunately, a pithed frog may remain responsive up to 5 days if kept at 4° C.

RESULTS

Fig. 1 illustrates the increased rate of dispersion when the second adrenaline is preceded by ergotoxine.

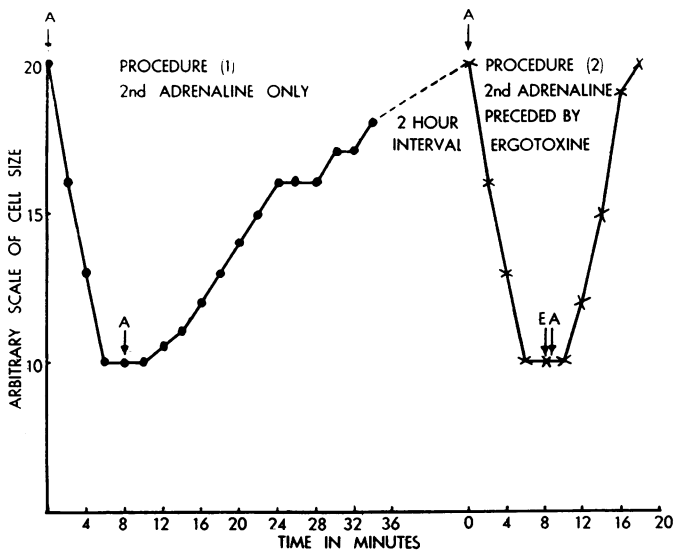

FIG. 1.—The graph illustrates the different rates of dispersion with procedures (1) and (2). A = Adrenaline 2 μg. E = Ergotoxine 35 μg./10 g. of frog.

The results of carrying out all the procedures described, in 24 different frogs, are summarized in the Table.

The mean time for the pigment to reach the arbitrary size of 20 is significantly less when ergotoxine precedes the second adrenaline injection than when ergotoxine

TABLE

TABLE GIVING THE TIME IN MINUTES TO REACH DISPERSION ARBITRARILY FIXED AT 20 BY VARIOUS DRUGS GIVEN 8 MINUTES AFTER INITIAL ADRENALINE

(Number of frogs used: 24)

Drug	Mean time (min.)
Initial adrenaline without further injection	32.1 (S.E.)±1.71
(1) 2nd adrenaline only	32.4 (S.E.)±1.07
(2) Ergotoxine and adrenaline	16.5 (S.E.)±0.55
(3) Ergotoxine only	21.6 (S.E.)±1.14
(4) Ergometrine and adrenaline	40.5 (S.E.)±1.15

Difference between procedures (2) and (3), $P < 0.001$

is given alone. This indicates that the pigment of the melanophore disperses more quickly with adrenaline after ergotoxine than with ergotoxine or adrenaline alone. However, ergotoxine alone also reduces the time taken to reach the arbitrary size of 20. Why ergotoxine alone should cause this dispersion is not clear. If ergotoxine is given when the pigment of the melanophore is dispersed it causes concentration, but if given when the pigment is concentrated, then occasionally a slow dispersion may be seen. Further, under these experimental conditions there may be enough circulating adrenaline after the initial dose to give a modified adrenaline reversal with ergotoxine alone, and this may account for the observed dispersing action of ergotoxine.

It is apparent from the Table that if ergometrine precedes the second adrenaline injection the time taken for the pigment to disperse (size 20) is increased.

In order to expand the scope of the investigation it was decided to repeat the experiment using noradrenaline (a racemic mixture was used). However, it was found that noradrenaline, in a dosage of 2–4 μg. intravenously, caused an initial concentration followed by a relatively rapid dispersion of the pigment.

Discussion

It is suggested that measuring the rate of dispersion of a concentrated melanophore is a suitable method of assessing whether or not a drug has a dispersing action on the melanophore.

The method reported here is open to subjective errors. While this is undoubtedly a serious objection, it is believed that with practice the observational error can be diminished. The objection that keeping frogs for several days may alter the responses of the melanophore is overcome by carrying out the procedures in a random order. The only difficulty encountered in keeping the frogs for several days is that about 25 per cent of them die before completion of the experiment.

Spaeth and Barbour (1917) and Shen (1937), from microscopic observations on the fish scale and the frog's web respectively, concluded that ergotoxine reversed the excitor action of adrenaline. The work reported here on single melanophore responses is in accordance with this previous work. Further, the fact that these observations were made on the same melanophore demonstrates that the mechanism is a reversal of the action of adrenaline and not an unmasking of an adrenaline inhibition.

SUMMARY

A melanophore was initially concentrated by adrenaline and the time taken to reach an arbitrary size was measured linearly by means of a scale in the eyepiece of a microscope. When the melanophore was concentrated, the following separate procedures were carried out: (1) adrenaline alone, (2) adrenaline preceded by ergotoxine, (3) ergotoxine alone, and (4) adrenaline preceded by ergometrine. All the drugs were injected intravenously.

It was found that adrenaline alone did not alter the rate of dispersion, but if ergotoxine preceded the adrenaline then the rate of dispersion was significantly increased when compared to the other procedures.

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