Histamine liberators and melanophores of *Rana tigrina*

SIR,—Adrenaline, noradrenaline and melatonin are known to turn frogs yellow by centripetal movement of melanin granules in the individual skin melanophores (Burgers & Van Oordt, 1962). We have now found that substances known to release histamine from mammalian tissues also turn conscious and anaesthetized frogs yellow.

Adult conscious *Rana tigrina* of either sex (weight 150–350 g) were observed in daylight in glass jars containing water. The frogs were treated with drugs dissolved in water and injected into the abdominal cavity.

Frogs which were anaesthetized were given sodium pentobarbitone (50 mg/kg) injected into the abdominal cavity. Drugs, dissolved in 0·6% saline, were injected through a polyethylene cannula tied into the left branch of the thoracic aorta. Histamine liberators which were comparatively less potent or painful on injection were administered only to anaesthetized frogs.

The skin colour was observed with the naked eye and the melanophores of the web skin of the hind limbs by microscope. Changes in distribution of melanin in melanophores were recorded in grades (Hogben & Slome, 1931).

Rana tigrina usually maintains a brown-black colour of the skin (melanophores of grade 5 or 4) during handling, injection or anaesthesia. Twenty-nine diverse substances (including digoxin, sodium cyanide, dibenzyline, ATP, oxytocin) which are not known to release histamine in the mammal did not affect the melanophores of the anaesthetized frog.

Histamine liberators turned conscious frogs deep yellow within 1 hr (Table 1). At this stage melanophores appeared under the microscope (× 60) as punctate spots (grade 1 or 2). Within 4–6 hr the frogs turned brown again. Throughout the experiment the animals appeared otherwise normal and healthy, and responded again if reinjected after 4 days' interval. In anaesthetized frogs, histamine liberators produced a similar change though in smaller doses (Table 1) and of shorter duration.

TABLE 1. DOSES OF HISTAMINE LIBERATORS WHICH PRODUCE MELANOPHORE CHANGES IN ANAESTHETIZED AND CONSCIOUS FROGS

Drug	Anaesthetized frogs, effective dose mg or ml/kg (No. of experiments)		Conscious frogs, effective dose mg or ml/kg (No. of experiments)	
Compound 48/80	0·01	(15)	2·0	(8)
Polymyxin B sulphate	0·05	(11)	2·5	(5)
Tubocurarine chloride	0·04	(10)	4·0	(6)
Propamidine isethionate	1·00	(13)	8·0	(12)
Polysorbate 80	0·003 ml	(22)	0·05 ml	(24)
Gallamine triethiodide	7·5	(7)	—	
Peptone	25·0	(8)	—	
50% v/v fresh egg-white solution	1·5 ml	(10)	—	
Horse serum	2·0 ml	(7)	—	
Russell's viper venom	1·00	(5)	—	
Trypsin	25·0	(7)	—	
Histamine acid phosphate	11·0	(9)	—	
Noradrenaline	0·5	(10)	—	
Adrenaline	2·0	(5)	—	

Also, histamine liberators were effective in decapitated frogs and when injected into subcutaneous lymph sacs of isolated limbs of anaesthetized frogs. This suggests a direct action on skin melanophores.

The doses of potent histamine liberators compare favourably with that of noradrenaline (Table 1). In 2 groups each of 10 anaesthetized frogs, dibenzyline (25 mg/kg) blocked the action of effective doses of noradrenaline and adrenaline (Table 1) on frog melanophores. In another group of 9 anaesthetized frogs dibenzyline failed to block the action of effective doses of histamine liberators

(tubocurarine, compound 48/80 and polysorbate 80) on melanophores. This suggests that the histamine liberators act on melanophores by a mechanism which is different to that of noradrenaline or adrenaline.

Histamine acted like the histamine liberators except that even with much higher doses (200 mg/kg as histamine acid phosphate) the degree of melanin concentration was less than that induced by histamine liberators. This dose of histamine was well-tolerated by the frogs which are known to be resistant to it (Rocha e Silva, 1955).

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β-Adrenergic auto-inhibition of the effect of noradrenaline on avian pulmonary artery

SIR,—We have previously reported that the predominantly β-adrenergic amine, isoprenaline, exerts both β-adrenergic vasodilator and α-adrenergic vasoconstrictor effects on isolated vascular smooth muscle (Somlyo & Somlyo, 1964, 1966a). In contrast, noradrenaline is a predominantly α-adrenergic vasoconstrictor amine, and its β-adrenergic vasodilator effects have previously been demonstrated only after α-adrenergic blockade, *in vivo* (Brick, Hutchison & Roddie, 1966). In the absence of α-adrenergic blockade, noradrenaline is one of the most potent vasoconstrictors of large and medium vessels (Somlyo, Sandberg & Somlyo, 1965a; Somlyo & Somlyo, 1966b) when potency is judged by maximum isotonic response. We now find that, in certain types of vascular smooth muscle, noradrenaline, in the absence of α-adrenergic blocking agents, can exert sufficient β-adrenergic vasodilator activity to produce auto-inhibition of the α-adrenergic vasoconstrictor effect.

Right and left main branches of the pulmonary artery were obtained from rapidly exsanguinated chickens. The preparation of helically-cut vascular strips and recording methods employed in our laboratory have been reported in detail (Somlyo & Somlyo, 1964; Somlyo, Sandberg & Somlyo, 1965a,b; Somlyo, Woo & Somlyo, 1965; Woo & Somlyo, 1966). The temperature for the present experiments was maintained at $41{\cdot}5 \pm 0{\cdot}5°$. Loading tensions applied were 2 g for pulmonary and 3 g for sciatic artery strips.

Fig. 1 shows the effect of the β-adrenergic blocking agent, pronethalol, on cumulative dose-response curves of pulmonary (1A) and sciatic (1B) artery strips to noradrenaline. Auto-inhibition of α- by β-adrenergic effect in pulmonary vascular smooth muscle is indicated by the maximum contractile effect, which is increased by β-adrenergic blockade, being depressed. Similar results were obtained in another group of 5 pulmonary arteries, suspended in Mg-free Krebs solution. The maximum isotonic response of the two pooled groups