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THE EFFECTS OF EXTRACTS FROM NEUROSECRETORY CELLS IN THE ANTERIOR VENA CAVA AND PHARYNGO-OPHTHALMIC VEIN UPON THE HEARTS OF INTACT FREE-MOVING OCTOPUSES

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SUMMARY

Recordings of pressure and frequency were made from the hearts of free-moving *Octopus vulgaris*. The effects of extracts from neurosecretory endings in the anterior vena cava (AVC) and the pharyngo-ophthalmic vein (POV), injected through fine cannulae into a branchial heart, efferent branchial vessel or the dorsal aorta, were studied and compared with the effects of acetylcholine, 5-hydroxytryptamine, adrenaline, histamine and tyramine. AVC and POV extracts each produce a different spectrum of effects, unlike those of any of the drugs tested. AVC extract is effective at doses of less than 2% of the material extractable from a single vein per kg, increasing the force and amplitude of the heartbeats. With a natural release point just upstream of the branchial hearts the AVC material must be relevant to the normal performance of the hearts. POV extract is effective only at doses equivalent to several veins per kg, and is unlikely to have a role in cardiac regulation. Section of the visceral nerves did not affect the action of drugs or extracts, indicating that effects were not indirectly mediated via the CNA. Further experiments were made with hearts and the aorta *in vitro* with effects that did not always parallel those found *in vivo*. Reasons for these differences are discussed.

INTRODUCTION

The effect of drugs upon the *Octopus* systemic heart has been studied mostly with isolated preparations (see Krijgsman & Divaris, 1955). Only one study has been made with intact free-moving octopuses, by Johansen & Huston (1962) using *O. dofleini*. These *in vivo* results were in some cases quite different from those found in the *in vitro* experiments. Since then neurosecretory cells have been found with discharge points in the anterior vena cava (AVC) (Alexandrowicz, 1964, 1965) and in the pharyngo-ophthalmic vein (POV) (Boycott & Young, 1956; Froesch, 1974). Both structures can be used to prepare extracts which excite the systemic heart

in vitro, causing increases in the frequency and amplitude of the heartbeat (Berry & Cottrell, 1970; Blanchi, 1969; Blanchi & De Prisco, 1971; Froesch & Mangold, 1976).

In this account the effects of AVC and POV extracts on the heartbeat of intact free-moving *Octopus vulgaris* are examined and compared with the actions of acetylcholine, 5-hydroxytryptamine, adrenaline, tyramine and histamine.

METHODS

(*a*) In vivo *experiments*

Experiments were made at Banyuls in July and August 1977. The animals used were *O. vulgaris*; healthy, feeding, mostly males, and of between 400 and 1700 g. Resting cardiac performance fell within the limits described by Wells (1979).

In most instances pressure was recorded from a point in the dorsal aorta, about 3 cm downstream of the systemic heart using an SE4-82 piezo-electric pressure transducer (SE Laboratories Ltd., Feltham, Middlesex), as described by Wells (1979). Injections were made through a fine polythene cannula (*ca.* 0·2 mm inside diameter) tied into the wall of one of the branchial hearts, so as to discharge into the lumen of the heart. Injected here, extracts of AVC or POV had to pass through the gills before reaching the systemic heart, and so records could reflect effects upon the gill capillaries and the branchial heart on the side concerned, as well as effects upon the systemic heart. It is, on the other hand, the route by which any hormonal secretion from the AVC or POV would have to come to the systemic heart; if the secretions of these organs are truly concerned in regulation of the systemic heartbeat, as the *in vitro* experiments would suggest, they must be effective when introduced through the branchial hearts.

Further experiments were made to evaluate the contribution of any changes in peripheral or gill capillary resistance, or in the beat of the gill hearts. In one series, injection was made into an efferent branchial, downstream of the gill capillaries, or into the aorta itself, downstream of the systemic heart. In each case pressure was recorded from the aorta. In a second series, pressure was recorded from an afferent branchial vessel following injection into the corresponding gill heart.

(*b*) In vitro *experiments*

Animals were killed by decapitation and a systemic heart preparation, consisting of the two auricles and a short length of the dorsal aorta, was dissected out. One auricle was tied around an inlet pipe, connected to a reservoir about 20 cm above the heart. About 3 cm upstream of the heart, there was a length of rubber tubing through the walls of which drugs and extracts could be injected. The other auricle was tied around a further pipe running to a tap used to adjust the rate of flow through the system. The auricles were pinned to a wax dish, and the stub end of the aorta tied to an isotonic lever writing on a conventional smoked-drum kymograph.

In five instances the direct action of drugs on blood vessels was investigated, using lengths of the dorsal aorta taken from animals freshly killed by decapitation. For these experiments, one end of a length of aorta (approximately 5 cm) was connected to the same T-piece and pressure-transducer as in the *in vivo* experiments. Beyond the T there was a stopcock instead of the peripheral circulation. The other

end of the aorta ran to a syringe via short length of rubber pipe through which drugs could be injected. This arrangement allowed one to inject a test dose, open the stopcock and push the dose along into the aorta, and then rapidly adjust pressure to any desired starting value before recording the effect of the drug on the walls of the aorta.

(*c*) *Extracts, drugs and dosages*

Anterior vena cavae and pharyngo-ophthalmic veins were dissected out from freshly killed animals, placed in distilled water and broken up in an MSE Sonicator, set at 15 μm for 5 min. The system was cooled with crushed ice around the tube, a precaution that is probably unnecessary, since both AVC and POV extracts appear to be heat stable; the activity of either will survive boiling (Berry & Cottrell, 1970; Froesch & Mangold, 1976). Sonicated extracts were centrifuged for 1 h at 4 °C and 5000 rev/min, the supernatant lyophilized and the dry extract taken up in filtered sterile sea water.

For *in vivo* experiments, dosages of extracts are given in units (vein per kg) that show how many AVC or POV were represented in the injection given, per kilogram of recipient. There is one AVC and there are two POVs in each octopus.

Drugs were all bought from Sigma Chemical Co., St Louis, Mo., U.S.A. Those used were: acetylcholine chloride, list No. A 6625; L-adrenaline, E-4250; 5-hydroxytryptamine, H-7752; histamine, H-7125; tyramine, T-7255. 5-HT was in a creatine sulphate complex, histamine and tyramine as free bases. Stock solutions were made with 20 mg of drug in 10 ml of filtered sterile sea water at room temperature, without acidification. Adrenaline was made up freshly as required. Other stocks were kept refrigerated for as long as 2–3 weeks, with no detectable decline in effectiveness. Dosages were made by dilution from stock solution, generally in about 0·2 ml of sea water.

RESULTS

(A) Effects upon the systemic heartbeat

1. *Extracts from the anterior vena cava (AVC)*

(*a*) Injected into one branchial heart and recorded at the dorsal aorta, the effect was always (one animal, 4 different dosages) a rise in the mean blood pressure and pulse amplitude accompanied by a fall in heartbeat frequency. The response depended upon the dosage, with injections of extracts made from as little as 0·03 of one vein per kg of recipient having an effect on all three parameters. Larger doses (0·08–0·15 of the extract from one vein per kg) induced changes lasting for 4 or 5 min (Fig. 1*a*).

(*b*) Very similar results were obtained when the extracts were injected into an efferent branchial vessel, downstream of the gills (13 experiments with 3 animals). Again, very small dosages (down to 0·01 vein kg^{-1}) sufficed to produce an effect on the heartbeat. With larger doses (0·08 and 0·16 vein kg^{-1}) the effects again lasted for several minutes, and included some reduction in heartbeat frequency (Fig. 1*b*).

(*c*) When AVC extracts were injected into the dorsal aorta, downstream of the systemic heart (11 experiments, with 3 animals), pressure and pulse amplitude again rose. At the highest doses there was also some decline in heartbeat frequency, but this

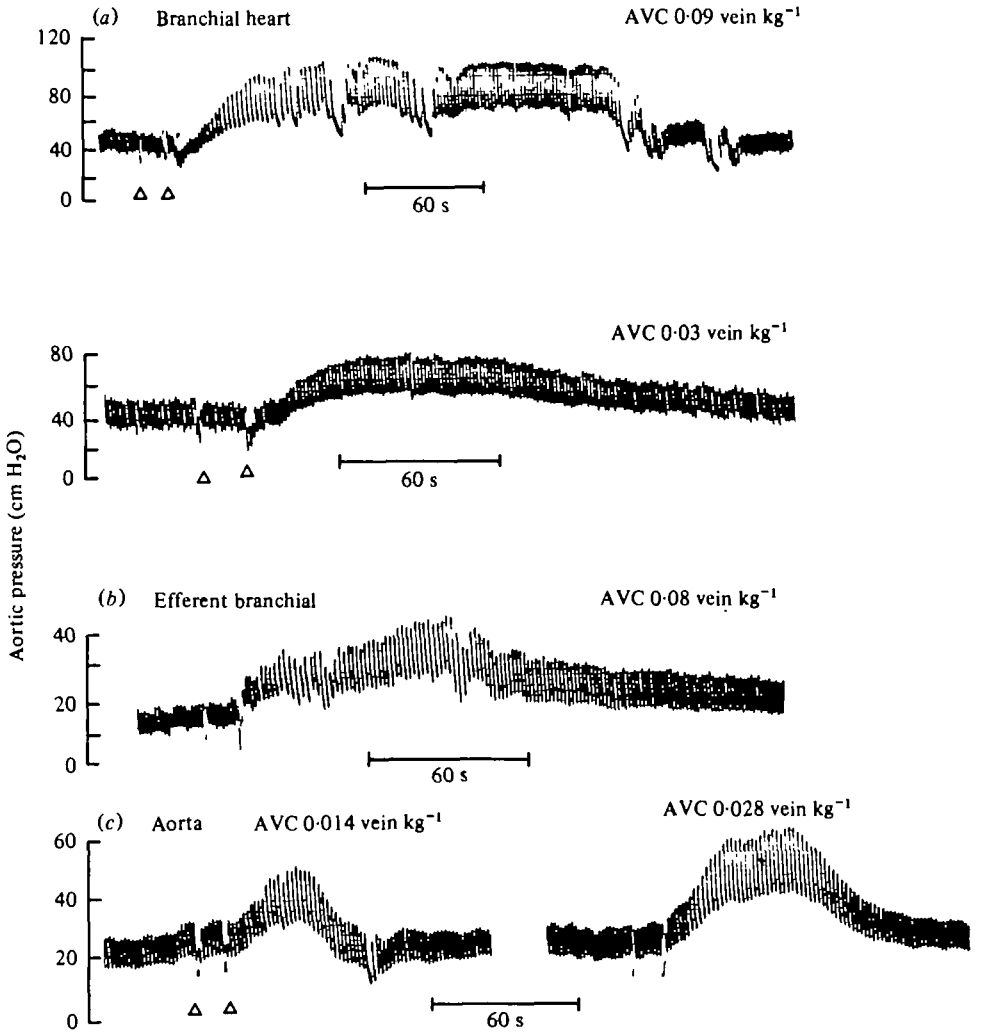

Fig. 1. Effects of AVC extracts (*a*) into a branchial heart at 0·09 vein kg^{-1} and 0·03 vein kg^{-1} (animal C53), (*b*) into an efferent branchial vessel at 0·08 vein kg^{-1} (C126) and (*c*) into the aorta at 0·014 and 0·028 vein kg^{-1} (C130). In each case Δ—Δ show the beginning and end of injection.

effect was much less marked than with AVC injected at levels where it could reach the systemic heart (Fig. 1*c*).

The responses found *in vivo* are thus quite different from those reported *in vitro* by Blanchi (1969) and by Berry & Cottrell (1970, with *Eledone*), both of whom showed considerable increases in heartbeat frequency, associated with increases in the amplitude of the beat. Our own *in vitro* experiments (one heart, 3 injections of 0·07 veins apiece) confirm theirs in showing in each case a rapid acceleration of the heart (beat frequency increases by *ca.* 20%) accompanied by a large (8 or 10×) increase in amplitude. Frequency returned to its resting value within 2–3 min, with amplitude remaining elevated for a little longer.

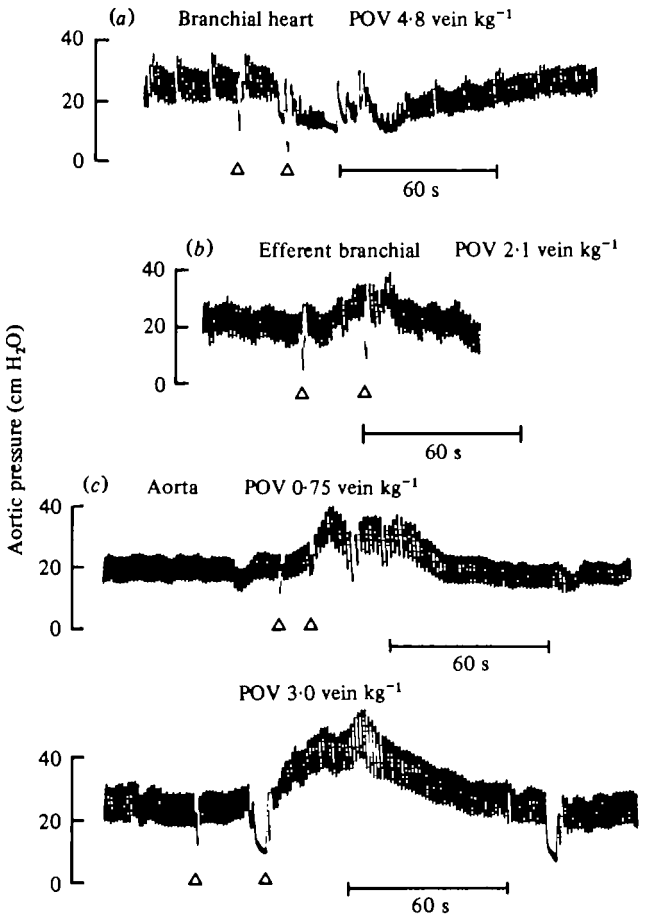

Fig. 2. Effects of POV extracts (*a*) into a branchial heart at 4·8 vein kg^{-1} (C25), (*b*) into an efferent branchial vessel at 2·1 vein kg^{-1} (C126, cf. Fig. 1*b*) and (*c*) into the aorta at 0·75 and 3·0 vein kg^{-1} (C78). Δ—Δ, beginning and end of injections.

2. *Extracts from the pharyngo-ophthalmic vein (POV)*

(*a*) Injected into a branchial heart, the effect of POV extract was always to *reduce* the mean pressure, pulse amplitude and frequency of the systemic heartbeat (17 experiments, with 3 animals). If the dose was large enough (> 2 vein kg^{-1}) inhibition was likely to be followed by a rise in pulse amplitude and mean pressure, which could remain above resting, pre-injection, levels for 2 or 3 min. Fig. 2*a* shows the effect of an injection at 4·8 vein kg^{-1}; there was no delayed rise in this instance.

(*b*) Injected into an efferent branchial, doses of from 0·5 to 2·0 vein kg^{-1} had little effect but slightly larger doses caused an increase in mean pressure (up to 2× at 3·7 vein kg^{-1}), with little effect on pulse amplitude or frequency (Fig. 2*b*) (12 experiments, with 2 animals).

(*c*) Injection downstream of the systemic heart regularly produced a rise in mean pressure, usually accompanied by some increase in pulse amplitude and sometimes by a modest fall in heartbeat frequency (Fig. 2*c*). Effective dosages were considerably

lower than those required further upstream; but 1 vein kg^{-1} or greater was normally required to induce a doubling of the mean aortic pressure (12 experiments with 3 animals).

The response *in vivo* is thus again quite different from that reported for the isolated heart, in this instance by Froesch & Mangold (1976) for *Eledone*. They found that the extract from a single POV per heart caused an increase in the amplitude of the heart beat, while larger doses (3 POV per heart) at first inhibited contraction and then not only increased the amplitude of the beat, but also evoked a long-lasting increase in beat frequency. Their results, including the increase in frequency, have been confirmed in the course of our own *in vitro* experiments, using extracts that failed to induce any signs of acceleration *in vivo* (9 experiments on 2 isolated hearts, using dosages of from 0·7 to 1·8 vein per heart).

3. 5-*Hydroxytryptamine*

5-Hydroxytryptamine excites and acetylcholine inhibits the heartbeat *in vitro* and it is generally believed that the pair are likely to be the natural excitatory and inhibitory transmitters in the hearts of most molluscs (see Hill & Welsh, 1966).

The effects of injections of 5-HT *in vivo* may be summarized as follows:

(*a*) Into a branchial heart (24 experiments, 7 animals). 2 $\mu g\ kg^{-1}$ and above increased mean pressure, pulse amplitude and beat frequency. Above 10 $\mu g\ kg^{-1}$ these effects were typically preceded by a temporary inhibition, as shown in Fig. 3*a*.

(*b*) Into an efferent branchial vessel (12 experiments, 2 animals). Similar effects to (*a*), at slightly lower dosages (1 $\mu g\ kg^{-1}$ and above, see Fig. 3*b*). 20 μg, the largest dose tested, drove the record off scale, with systolic pressures of 70 cm H_2O^+, and almost doubled the heartbeat frequency.

(*c*) Into the aorta (17 experiments, 5 animals). Increases in pulse amplitude and mean pressure, but no effect on beat frequency (Fig. 3*c* shows the effect of a dose at 4 $\mu g\ kg^{-1}$).

In contrast to the results with AVC and POV the effects of 5-HT upon the systemic heart *in vivo* were exactly those to be expected from *in vitro* experiments with isolated hearts. Our own *in vitro* preparations (15 experiments, doses of from 1 to 3 μg on a total of 4 different hearts), like those reported in the quite extensive literature (see Krijgsman & Divaris, 1955), invariably showed an increase in beat amplitude and frequency in response to 5-HT.

4. *Acetylcholine*

Effects of injections of ACH *in vivo* were as follows:

(*a*) Through a gill heart (25 experiments, with 7 animals). Doses of from 20 to 100 $\mu g\ kg^{-1}$ caused rapid falls in mean pressure, pulse amplitude and beat frequency; typically followed, after a minute or more, by rises in pulse and pressure that could last for 2 or 3 min and could greatly exceed pre-injection resting levels (Fig. 3*d*).

(*b*) Into an efferent branchial (5 experiments, 2 animals). Very similar effects to (*a*) were induced by injections downstream of the gills (doses of from 15 to 50 $\mu g\ kg^{-1}$). The inhibition caused by ACH (unlike, for example, that arising from POV extracts) is evidently a direct effect upon the heart and not due to vasoconstriction in the gills.

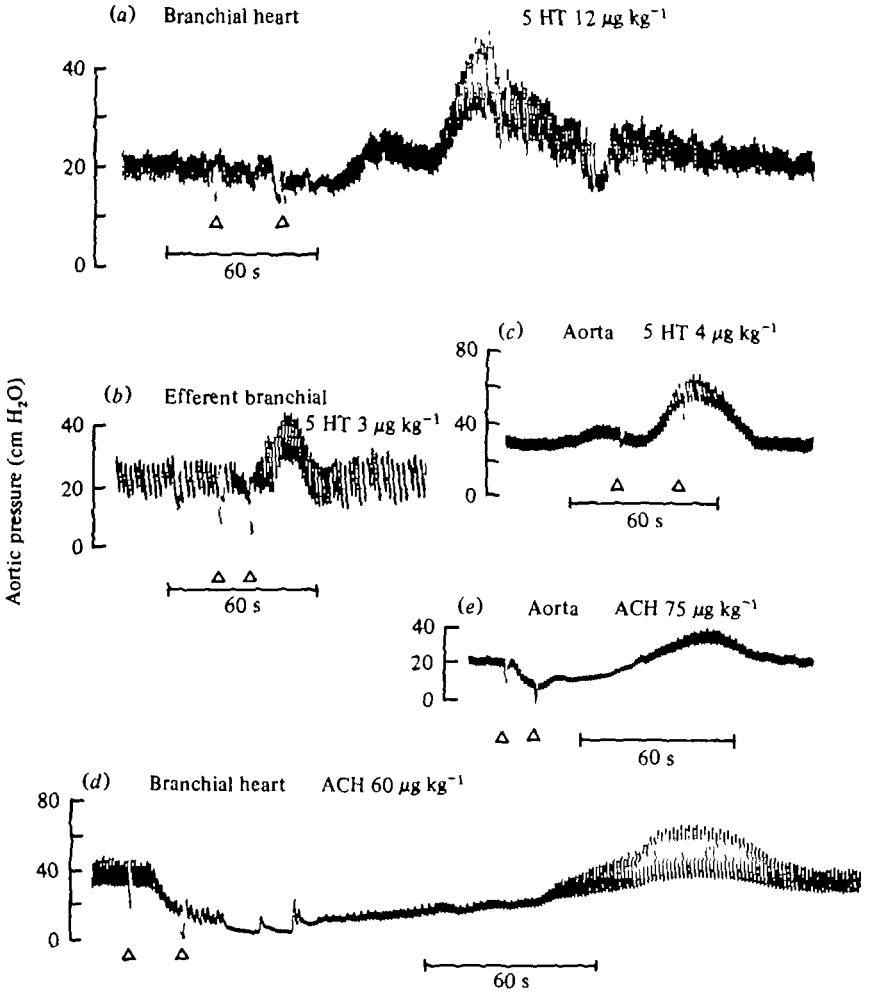

Fig. 3. 5-Hydroxytryptamine and acetylcholine. (*a*) 12 μg kg^{-1} 5-HT injected into a branchial heart; a dose as high as this evidently produces some vasoconstriction of the gill capillaries, so that a period of apparent inhibition precedes the normal increased beat (animal C6). (*b*) 3 μg kg^{-1} 5-HT into an efferent branchial vessel (C126). (*c*) 4 μg kg^{-1} 5-HT into the aorta (C60). (*d*) Acetylcholine at 60 μg kg^{-1} injected into a branchial heart. Record shows inhibition followed after about 2 min by a prolonged overshoot in which the pulse is greatly increased for a period; similar effects seem to follow any period of reduced heartbeat (C38). (*e*) ACH at 75 μg kg^{-1} into the aorta; vasodilation and delayed overshoot (C60).

(*c*) Into the aorta (20 experiments, 6 animals). Vasodilation, with falls in pulse and pressure while frequency remained constant – doses of 50–100 μg kg^{-1}. The overshoot, observed to follow inhibition of the heart in (*a*) and (*b*) above, was also found to follow periods of reduced pressure in the aorta (Fig. 3*e*).

The effects of acetylcholine *in vitro* are well known (see Krijgsman & Divaris, 1955), and have been repeated by ourselves. The invariable result in our experiments (9 experiments, 4 animals, doses of 10–20 μg per heart) was inhibition of the heartbeat – generally the heart stopped altogether for 10–20 s – followed by recovery. There was no sign of the post-inhibitory overshoot, found in the *in vivo* experiments.

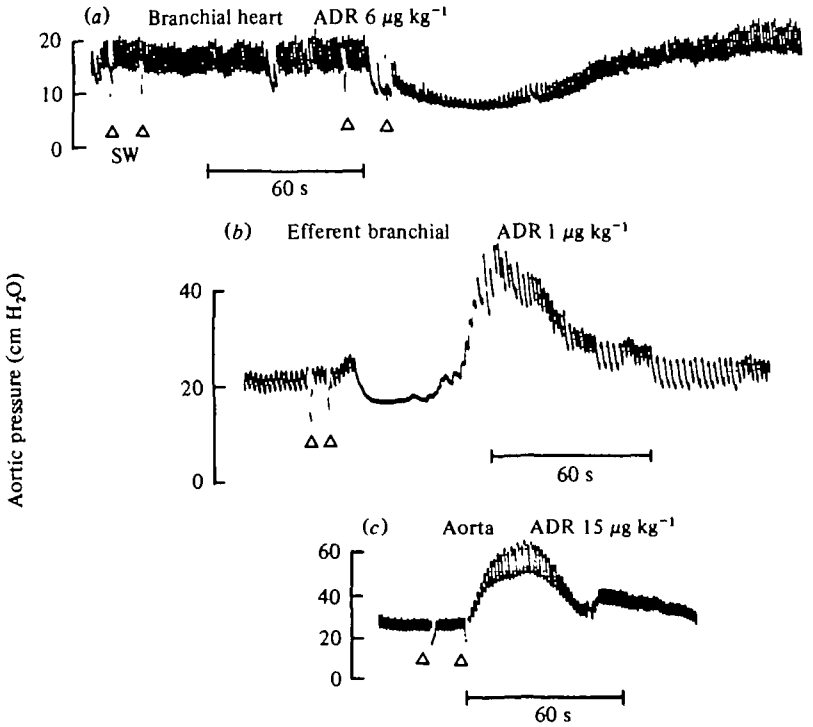

Fig. 4. Adrenaline. (*a*) 6 μg kg^{-1} injected into a branchial heart. At the start of this trace the same volume (0·5 ml) of sea water was injected (C25). (*b*) 1 μg kg^{-1} into an efferent branchial (C132). (*c*) 15 μg kg^{-1} into the aorta (C60).

5. *Adrenaline*

In vivo:

(*a*) Injected into a branchial heart (31 experiments, 5 animals). Doses from 2 to 20 μg kg^{-1} slowed the heart and reduced pulse and pressure in the aorta (Fig. 4*a*). 100 μg kg^{-1} – given in error – stopped the heart of an animal for about 1 h, with subsequent recovery!

(*b*) Into an efferent branchial (3 experiments, 1 animal). 1–2 μg kg^{-1} stopped the heart; in each instance resumption of the heartbeat was accompanied by large but short-lasting increases in pulse and pressure (Fig. 4*b*).

(*c*) Into the aorta (8 experiments, 2 animals). Immediate rises in pulse and pressure followed injection of doses in the range 10–60 μg kg^{-1}. This effect on peripheral resistance clearly distinguishes adrenaline from ACH, which produces an abrupt fall in resistance when injected downstream of the systemic heart.

In vitro, doses of 1–20 μg per heart all increased the amplitude and the frequency of the heartbeat (9 experiments, 3 hearts). This again confirms existing reports (see Krijgsman & Divaris, 1955).

6. *Tyramine*

In vivo:

(*a*) Into a branchial heart (25 experiments, 5 animals). Inhibition; 10–100 μg kg^{-1} slowed, and at the highest doses, stopped the heart. As with ACH, and POV,

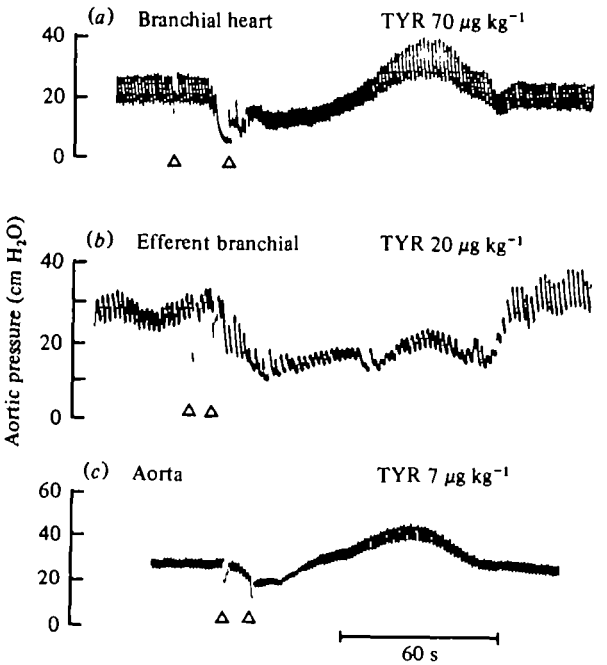

Fig. 5. Tyramine. (*a*) 70 μg kg^{-1} into a branchial heart (C53, a rather insensitive animal). (*b*) 20 μg kg^{-1} into an efferent branchial (C132). (*c*) 7 μg kg^{-1} into the aorta (C60).

inhibition was followed by an overshoot in which pulse and pressure rose above their pre-injection levels (Fig. 5*a*).

(*b*) Into an efferent branchial (4 experiments, 1 animal). Similar effects to (*a*); inhibition is not due to strangulation of the blood supply to the systemic heart (Fig. 5*b*).

(*c*) Into the aorta (10 experiments, 3 animals). Vasodilation with doses at 3–40 μg kg^{-1}; pulse and pressure dropped while frequency remained constant, as with ACH. Once again there was a delayed overshoot.

Again we have a marked difference in the effect of a drug on the heart *in vivo* and *in vitro*. Tyramine, like adrenaline, is excitatory *in vitro*: the isolated heart increases the amplitude and the frequency of its beat (2 experiments with one heart at 20 μg per heart).

7. *Histamine*

A small number of experiments was made with histamine. Injected into the gill heart, two doses of 100 μg kg^{-1} caused modest (about 50%) rises in mean pressure and pulse amplitude, and a drop (38 to 32 beats min^{-1}) in heartbeat frequency. A 10 μg kg^{-1} dose had no effect.

Injected downstream of the systemic heart, two doses of 10 μg kg^{-1} and one of 20 μg kg^{-1} caused increases (doubling) in mean pressure, accompanied by a small increase in pulse amplitude, but no change in beat frequency. Evidently the main effect of histamine is vasoconstriction.

In vitro, histamine at 10 μg per heart caused large increases in beat amplitude and frequency in the two tests that we carried out.

(B) Effects on the branchial hearts

Any active product of the AVC or POV would enter the hearts system through the branchial hearts, so the effect of extracts from these tissues on the branchial hearts themselves is relevant to any analysis of the effect on the circulation as a whole. It has been shown above that POV, in particular, has quite different effects on the aortic pulse when injected upstream and downstream of the gill capillaries, apparently being inhibitory in the former case and excitatory in the latter. The most obvious explanation for the apparent inhibition was strangulation of the blood supply to the systemic heart by contraction of the gill capillaries, but it is also possible that POV extract directly inhibited the gill hearts.

To test this, and the effect of other drugs and extracts, five preparations had cannulae placed in the afferent branchial vessel on the same side as the finer cannula used for injection. In two of these instances the cannula was placed in the gill tip, facing towards the heart, and blocking the distal third of the gill capillaries on that side. In three other instances the cannula passed through the heart itself from behind, alongside the finer tube used to inject drugs. Because the cannulae must inevitably have restricted gill and heart movement a little, this treatment may have reduced the absolute pressures recorded (they are lower than those recorded for *O. dofleini* by Johansen & Martin (1962)), but changes in beat amplitude and frequency were easily detected.

The pressure changes in general resembled those obtained for the systemic heart, measured in the aorta following injections of drugs into an efferent branchial.

Thus AVC extracts always caused increases in pulse and mean pressure (11 tests with 2 animals, doses of 0·03–0·17 vein kg^{-1}, see Fig. 6*a*), sometimes (4 out of the 11 cases, all with the same animal, at doses of 0·07–0·15 vein kg^{-1}) following an initial reduction in amplitude of the beat.

POV extracts had little effect; doses of 1·4 and 1·9 vein kg^{-1} increased the pulse and mean pressure in one animal (in which 0·48 vein kg^{-1} failed to do so), while a larger dose of 2·7 vein kg^{-1} (from, however, *Eledone* not *Octopus*) was without effect upon a second.

5-HT usually increased the pulse and, to a more limited extent, mean pressure (Fig. 6*b*) (16 tests, 6 animals, doses of 2–30 $\mu g\ kg^{-1}$; in 7 further instances, with 3 of the same animals, doses of 1–20 $\mu g\ kg^{-1}$ were ineffective).

ACH was always inhibitory (Fig. 6*c*, *d*) (12 tests with 2 animals, doses of from 10 to 200 $\mu g\ kg^{-1}$); with the larger doses the heart often stopped. With four exceptions (low doses of 10–20 $\mu g\ kg^{-1}$) there was a subsequent increase in the pulse, resembling the overshoot seen in recordings from the systemic heart made at aortic level (Fig. 6*c*). The initial reduction in pulse differed from that induced by AVC extracts in that it was always associated with a fall in mean pressure; AVC-induced inhibition of the beat centred around a mean pressure that tended to remain constant.

(C) Recordings from isolated aortae

The arteries of *Octopus* are wrapped in longitudinal and circular muscles and both layers are innervated, down to the finest branches (Alexandrowicz, 1928; Barber & Graziadei, 1965, 1967*a*, *b*). Drugs could affect peripheral resistance by acting on

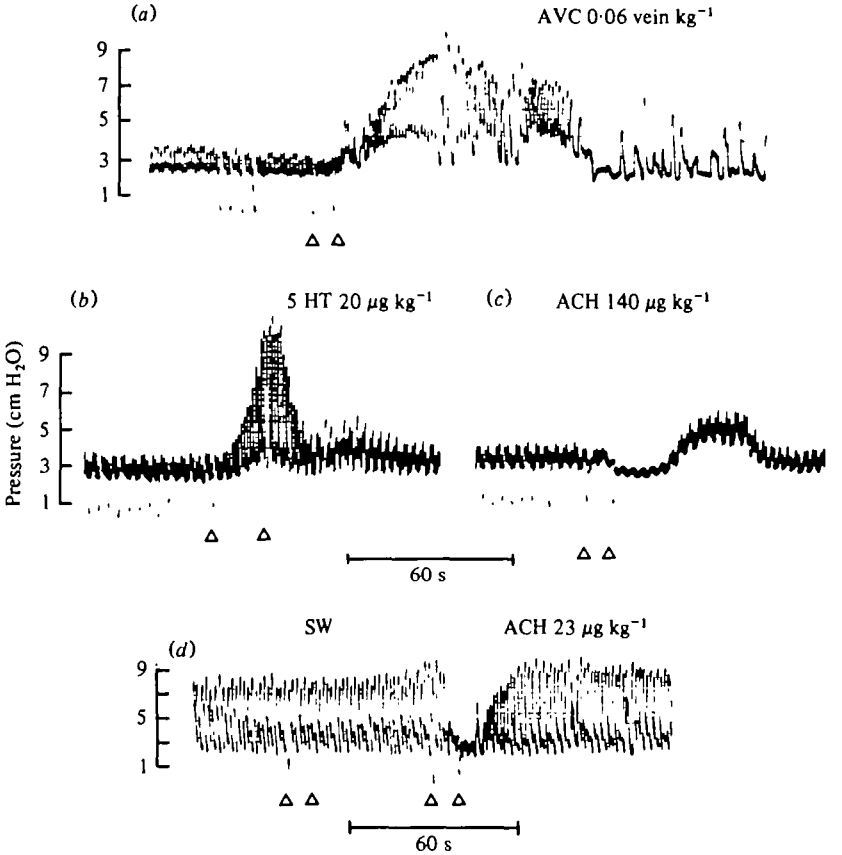

Fig. 6. Pressure records from afferent branchial vessels. (*a*) 0·06 veins kg^{-1} AVC extract; in the terminal part of this record the octopus began to move down the tank, distorting the pressure line; the four deflexions early in the record mark the respiratory rhythm. (C72). (*b*) 20 $\mu g\ kg^{-1}$ 5-HT, same animal. (*c*) 140 $\mu g\ kg^{-1}$ ACH, same animal. The row of dots in the early parts of records (*b*) and (*c*) show respiratory movements; at this degree of amplification these can be seen to produce pressure waves superimposed on the pulse. (*d*) 23 $\mu g\ kg^{-1}$ ACH in a second animal (C136) showing a larger resting pulse. The earlier part of this record shows the lack of effect of injecting the same volume of sea water.

the network of nerves around the vessels, or less directly through the central nervous system.

In an attempt to evaluate possible direct effects on blood vessels, aortae were set up as described in Methods. If inflated to a pressure of 25 cm or so (values close to the mean found in the heartbeat of intact octopuses apparently at rest, Wells, 1979) an aorta will maintain pressures in the physiological 'resting' range for many minutes. There are often spontaneous local contractions and relaxations, visible to the naked eye and reflected in the pressure record; these changes are always much slower than the heartbeat.

To test the effect of drugs one must use aortae that are minimally active. Effects such as those shown in Fig. 7 can then be observed. Of all the drugs and extracts tested on a total of five aortae, only 5-HT and ACH produced consistent effects (contraction and relaxation, respectively) at dosages as low as those found effective

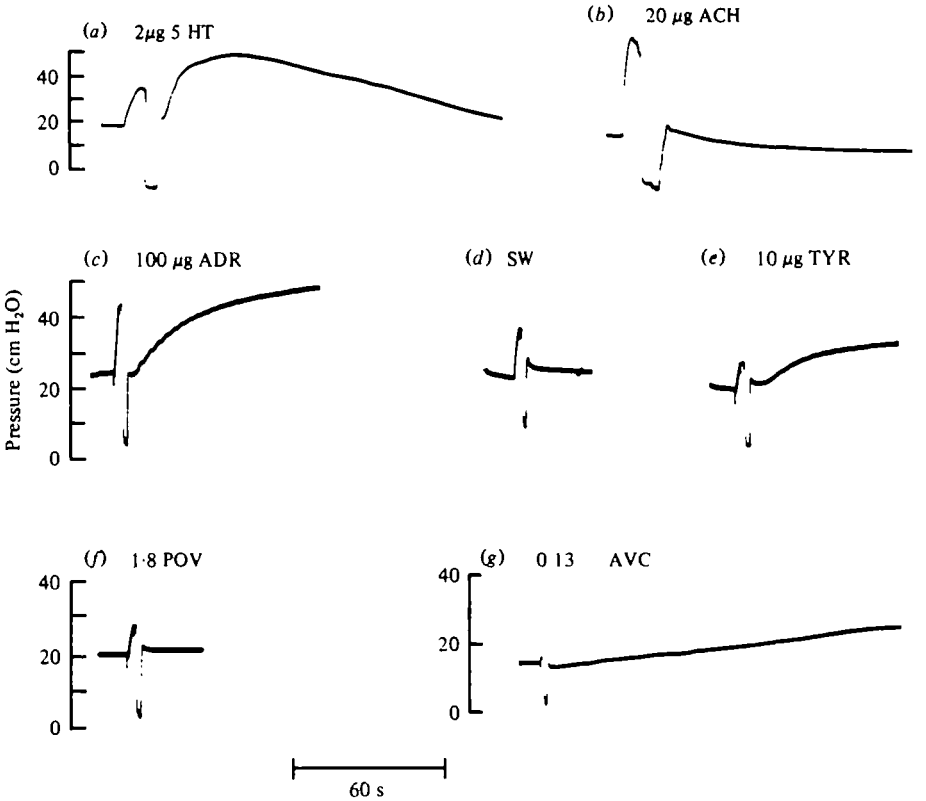

Fig. 7. Pressure changes in isolated aortae. In each case the drug, sea water or extract was injected into a cannula upstream of the aorta (rise in pressure) and allowed to flow into the aorta (stopcock at the far end opened, pressure falls and is restored by closing the stopcock and adding sea water by means of a syringe upstream of the injection point). Records show the effects of injecting (*a*) 2 μg 5-HT and (*b*) 20 μg ACH (animal C126), (*c*) 100 μg adrenaline, (*d*) sea water only, (*e*) 10 μg tyramine and (*f*) an extract of 1·8 POVs (all with an aorta derived from animal C141). (*g*) The effect of an extract of 0·13 AVC (C140).

on the heartbeat (Fig. 7*a*, *b*). Tyramine caused a fall followed by a rise in pressure in two aortae (at 1 or 2 μg per aorta, Fig. 7*e*) and falls alone in a third (20–200 μg per aorta, 2 μg per aorta having no effect at all). A large dose of adrenaline (100 μg per aorta) induced a jerky contraction in one out of two aortae (Fig. 7*c*); five smaller doses were without effect, and 200 μg per aorta failed to elicit a response in a second preparation. Histamine was ineffective at 1 and 10 μg per aorta, but caused a steady slow rise in pressure at 100 μg per aorta in the single aorta on which it was tested.

POV was without effect (5 tests at dose rates of 0·8 and 1·8 vein per aorta on two aortae) while AVC (at 0·13 vein per aorta) induced slow contractions in only one out of three preparations tested.

If one assumes that the performance of the aorta is a useful indication of direct effects on the peripheral vessels, the erratic results obtained and high dose rates required can only imply that the very considerable and regular effects of substances like adrenaline, and the secretions from the AVC and POV *in vivo*, are mediated through central nervous control of the blood vessels.

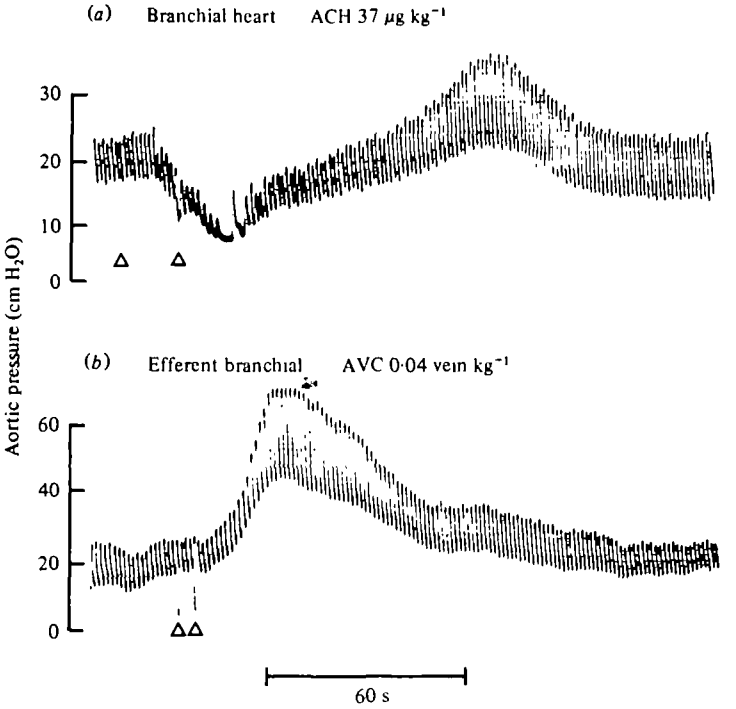

Fig. 8. Effects after section of the visceral nerves. (*a*) The effects, including delayed overshoot of 37 μg kg^{-1} ACH injected into a branchial heart (C25) and (*b*) 0·04 vein kg^{-1} AVC, into an efferent branchial vessel (C130).

(D) A control for central nervous effects upon the hearts

The hearts of *Octopus* are innervated through two pairs of ganglia, the fusiform and the cardiac ganglia. Both are linked to the CNS by the paired visceral nerves (Young, 1967). Drugs injected into any part of the hearts system will reach the brain within 10 or 15 strokes. This means, for example, that the pressure overshoot, so often observed to follow a period of inhibition, could well be (at least in part) a central nervous effect on the cardiac output, mediated through the visceral nerves. To check this possibility the visceral nerves were cut (usually after tests with the intact animal) just centrally to the fusiform ganglia in seven preparations. Most of the tests described above were repeated with the animals in this condition. In no case was the effect of a drug or an extract altered by severing the visceral nerves. Fig. 8 shows two examples; (*a*) summarizes the effect of introducting acetylcholine into a branchial heart; (*b*) the effect of an injection of AVC extract into an efferent branchial vessel.

DISCUSSION

The experiments outlined above were designed to find out whether the products of the neurosecretory cells in the AVC and/or the POV are likely to be of physiological significance in the intact animal, as well as cardioexcitatory *in vitro*.

These questions can now be answered. The product of the AVC is effective at very low doses, down to less than 2% of the material extractable from a single vein per kg of animal. With a release point just upstream of the branchial hearts, it can hardly fail to be relevant to the normal operation of the hearts. Blanchi (1969) noticed that while blood from the vena cava was cardioexcitatory *in vitro*, that from the arms 'molto rapidamente recisi' was not. The implication is that dragging the animal from its tank and opening it up promotes secretion into the vena cava, which has no time to get through to the arms if one is quick about it. Secretion in times of stress would make biological good sense, increasing cardiac output in 'fight or flight' situations, an analogue of mammalian adrenalin in a mollusc.

The product of the POV can have no such functions. Extracts of this material, prepared in the same way as those from the AVC, were effective only when injected at doses representing several veins per kg. The animal has two POVs, of course (unlike the unpaired AVC), but these are distant from the hearts. Any product released into the POV must run into the perioesophageal sinus and back around the gut before passing through the abdominal veins to join the vena cava. There are sphincters between the veins and the large perivisceral blood sinus. Any product would thus inevitably be much diluted, and probably much delayed, before reaching the hearts. Previous reports (Froesch & Mangold, 1976) have stressed the activity of extracts per milligram of source tissue and in these terms POV is a very active material. But the POV is very small compared with the AVC and so far as the whole animal is concerned it is the quantity of product per animal that matters. In these terms the POV emerges as most unlikely to be relevant to the heartbeat, or to the main peripheral circulation. More probably, since its principal effect seems to be vasoconstrictive, it is concerned in some way with the regulation of blood flow from the orbit, as Boycott & Young (1956) have suggested from the anatomy of the veins and sinuses.

Neither AVC nor POV has a spectrum of effects quite like that of any of the drugs tested.

In vitro, AVC resembles 5-HT, accelerating and increasing the beat amplitude of the isolated systemic heart. *In vivo* AVC slows the heart. The two actions are not incompatible. *In vitro* heart action is not limited by fluid input, or by a build-up of pressure on the output side. *In vivo* neither the supply nor the load is constant. If the action of AVC is to increase the force and amplitude of the systemic heartbeat, it would tend to speed up the heart *in vitro* and slow it *in vivo* since the rate of the systemic heartbeat is limited *in vivo* by the rate at which it can be filled. The specific accelerating action of 5-HT, which is perhaps attributable to a lowering of the threshold of receptors initiating the reflex response to distension, seems to be lacking in AVC extracts which must include a second, different, cardioexcitatory and vasoconstrictive substance, or mixture of substances.

The action of POV extract has not been so well defined in the present series of experiments, partly because it had to be delivered in such high doses to have any effect at all. It does appear to be cardioexcitatory *in vivo* as *in vitro*, increasing the pulse and raising the mean pressure when injected into an efferent branchial (Fig. 2*b*). But the effect is very slight compared with its effect on vasoconstriction (Fig. 2*c*), so that injection upstream of the gills actually appears to inhibit the heart. It seems

unlikely that we are dealing with the same substance as that extracted from the AVC and, again, rather improbable that the POV product has any relevance to the performance of the hearts, since vasoconstriction would, presumably, limit passage of the product from the POV into the perivisceral sinus and from there into the abdominal veins.

The results with acetylcholine, 5-HT, adrenaline, tyramine and histamine obtained in the present study closely resemble those already reported for *O. dofleini* by Johansen & Huston (1962); when the relative size of the two species is considered (*O. dofleini* is 10× the weight of *O. vulgaris*) it is evident that very similar dosages are effective in the two species. There is, however, one difference in interpretation that should be noted. Johansen and Huston report that the main action of adrenaline is vasodilation, and this is plainly not the case in *O. vulgaris*. Their conclusion seems to be based on the fall in diastolic pressure that follows injection of adrenaline into the efferent branchial vessel, an effect also shown by ourselves (compare their figs. 2 and 4 with our own Fig. 4). A fall of this sort, however, could equally well arise from a direct effect on the heart itself and this seems the more likely explanation in view of the effect of injection downstream of the heart, an experiment apparently not made with *dofleini*.

The origin of the delayed large rise in pulse and pressure that tends to follow any inhibitory effect is unresolved at present. Typically, it begins a minute or more after injection and may last for several minutes. The observation that it is characterized by a great increase in pulse amplitude without much increase in diastolic pressure (see Figs. 3, 5 and 8) implies that we are dealing with an increase in cardiac output rather than an increase in peripheral resistance. Qualitatively similar effects have been reported to follow stimulation of a visceral nerve *in vitro* (nerve plus isolated systemic heart preparations, Fredericq & Bacq (1939)) where the sequence of inhibition followed by excitation has been attributed to the simultaneous release of both excitatory and inhibitory transmitters; the latter is seen as reaching threshold levels first and decaying more rapidly. But the delayed increase in pulse amplitude seen *in vivo* is not prevented by section of the visceral nerves (Fig. 8*a*) and it is possible that the effects observed *in vivo* are unrelated to those seen in the isolated heart. Similar large increases in pulse amplitude can be seen when *Octopus* is returned to fresh sea water after a period in O_2-depleted water, in recovery from anaesthesia, and following exercise (Wells, 1979). It seems possible that the delayed overshoot seen in the intact animal is of the nature of a compensatory reflex, associated perhaps with the release of blood from the large capacity sinus around the gut, and driven, presumably, by the oxygen lack that must accompany any period of cardioinhibition.

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