THE EFFECT OF POSTERIOR LOBE PITUITARY EXTRACTS ON THE BLOOD PRESSURE OF *ORNITHORHYNCHUS* (DUCK-BILLED PLATYPUS)

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(With Four Text-figures)

I. INTRODUCTION

Whole extract of posterior lobe pituitary has several pharmacological properties (Waring & Landgrebe, 1950). Three of these are relevant to the present theme:

(*a*) Intravenous injection into spinal or fully anaesthetized eutherian mammals evokes a *rise* of blood pressure.

(*b*) Intravenous injection into spinal or anaesthetized fowls or ducks evokes a *fall* of blood pressure.

(*c*) After injection by any route (or *in vitro*) the uterus contracts.

Since no chemically pure excitant has been extracted for certain* from the posterior lobe, activity of a sample is expressed in International Units which are arrived at by direct comparison of the unknown extract with International Standard powder on a generally agreed biological preparation. Here it need only be noted that 'pressor' units are assayed on spinal or anaesthetized mammals, and 'oxytocic' units are assayed on the guinea-pig uterus *in vitro*.

By definition, 0·5 mg. International Standard powder contains 1 unit of oxytocic and 1 unit of pressor activity. In what follows, reference to pressor and oxytocic units are strictly to these activities as measured under the conditions specified in Mem. 36 and does not refer generally to blood-pressure raising, or uterus-contracting properties.

By fractional precipitation, or fractional absorption and elution, two fractions can be obtained from a simple whole extract. One, variously called oxytocin, pitocin, post lobin O, has oxytocic properties contaminated with 3–5% pressor activity. The other, variously called vasopressin, pitressin, post-lobin V, has pressor properties contaminated with 3–5% oxytocic properties.

Of the effects listed above (*a*) is evoked by pitressin and not to any measurable extent, by pitocin, (*b*) is evoked by pitocin predominantly (see p. 56) and (*c*) is evoked by pitocin; pitressin has no effect on the uterus *in vitro* unless there is magnesium in the saline. Since histamine, a common contaminant of pituitary extracts causes contraction of the uterus and depression of the blood pressure, it is

* It is possible that Van Dyke's preparation (1942) is pure, but this is not generally available.

relevant to note that the above observations have been made using histamine-free extracts.

The mammalian pressor response results from peripheral vasoconstriction, and experiments involving gross exclusion of various organs show that the effect is not localized to any particular organ or organs. Moreover, since some peripheral vessels constrict while others dilate, this implies that the pressor effect is not a simple one, and, of course, the orthodox measurement with a cannula in the carotid gives only an over-all figure. Generally speaking, pituitrin has no general effect on the heart, so the rise in pressure must be attributed to the heart's increased response to the load imposed by peripheral vasoconstriction. Paton & Watson (1912), who were the first to describe the avian depressor response, showed by oncometer recordings that it was accompanied by vascular dilatation in the splanchnic area, but Hogben & Schlapp (1924) showed that the response was still exhibited when this area was excluded from circulation.

Although much work remains to be done on the exact mechanism of the responses evinced by birds and mammals, the existence of such a gross difference in their over-all response to whole posterior lobe, and its separated fractions, is challenging with regard to the phyletic distribution of the two responses. Work shortly to be published will show that in some living reptiles, there is a pressor response to pitressin, a depressor response to pitocin and a depression to whole extract; the same is substantially true for the bird. So, in attempting a phyletic formulation comparable observations on a prototherian are of pivotal importance.

II. EXPERIMENTAL

Blood pressure was recorded in the usual way with a mercury manometer and a cannula inserted in the carotid. Injections were made into the femoral vein, each injection being washed in with 1 c.c. of saline. Control injections of saline were also 1 c.c. Heparin was used as anticoagulant in the animal and citrate in the manometer.

Two males and one female were obtained, each weighing between 2 and 3 kg. Each was prepared so as to exhibit a different level of central nervous control with the object of obtaining the maximum information for comparison with known data from birds and eutherians. As has been indicated, the degree of central nervous control in eutherian preparations is correlated only with quantitative changes in the response evoked. In the bird, however, we have reason to suspect that depth of anaesthesia, pithing, etc., may to some extent determine the qualitative nature of the response (see discussion later). The three preparations were therefore designed to produce the following states of central depression: (*a*) anaesthetized, respiratory centre functional (preparation 1); (*b*) anaesthetized, respiratory centre inhibited (preparation 3); (*c*) spinal (preparation 2).

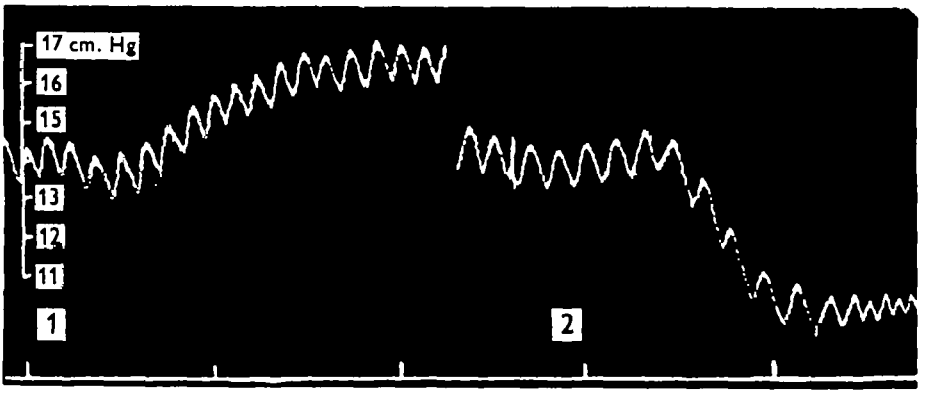

Fig. 1. Preparation 1. 1, 0·5 i.u. of pitressin; 2, 0·5 i.u. of pitocin. Time marks at minute intervals.

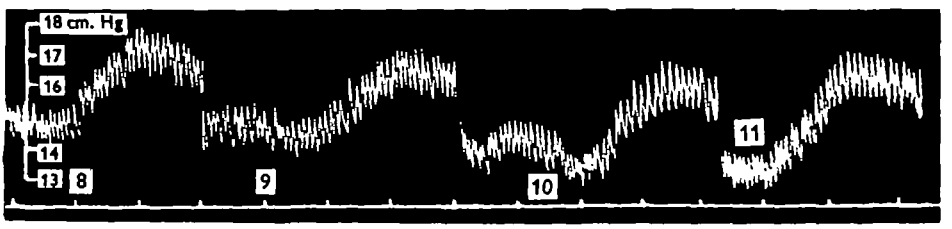

Fig. 2. Preparation 1. 8, 0·6 i.u. pitressin; 9, 0·6 i.u. pitressin; 10, 0·6 i.u. pitressin; 11, 1·2 i.u. pitressin. Time marks at minute intervals.

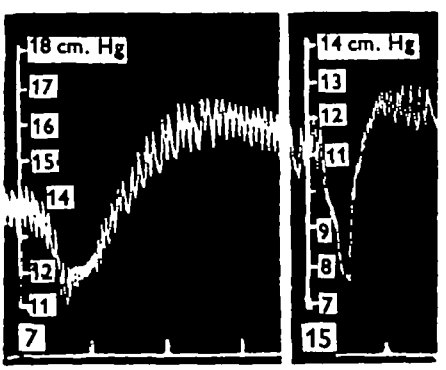

Fig. 3. Prepartion 1. 7, 0·6 i.u. pitressin + 0·6 i.u. pitocin; 15, unfractionated posterior lobe pituitary extract. Time marks at minute intervals.

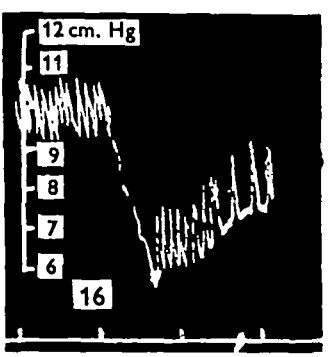

Fig. 4. Preparation 1. 16, 0·05 mg. (approx.) histamine acid phosphate. Time marks at minute intervals.

Preparation 1. *Male*—21 *January* 1949—*working sheet*

Animal anaesthetized with intraperitoneal Dial and urethane; vagi severed; trachea cannulated; no artificial respiration.

Time	Ref. no.	Injection	Response	Increment or Decrement (cm.)	Notes
17·45	—	0·25 c.c. 'Dial' (Ciba liquid)	—	—	Intraperitoneal injection
18·40	—	0·1 c.c. 'Dial' ,, ,,	—	—	—
20·30	—	200 mg. urethane	—	—	—
20·45	—	—	—	—	Dissection commenced
21·00	—	200 mg. urethane	—	—	—
21·15	—	200 mg. urethane	—	—	—
21·40	—	0·1 c.c. 'Dial'	—	—	—
22·05	—	—	—	—	Cannulae inserted
22·10	—	heparin	—	—	Intravenous injections
22·12	—	1 c.c. 0·9 % saline*	13·0–13·5	Up 0·5	Drum speed 48 mm./min.
23·50	1	0·5 i.u. pitressin	13·0–16·0	Up 3·0	—
00·07	2	0·5 i.u. pitocin	13·0– 9·4	Down 3·6	—
00·24	3	0·6 i.u. pitocin	13·0–10·6	Down 2·4	Drum speed 10 mm./min.
00·34	4	0·6 i.u. pitressin	13·0–15·8	Up 2·8	—
00·48	5	0·5 i.u. pitocin	13·0–10·8	Down 2·2	—
		+0·5 i.u. pitressin	13·0–15·4	and up 2·4	—
01·07	7	0·6 i.u. pitocin	12·6–10·4	Down 2·2	—
		+0·6 i.u. pitressin	12·6–15·6	and up 3·0	—
01·15	8	0·6 i.u. pitressin	14·0–16·2	Up 2·2	—
01·24	9	0·6 i.u. pitressin	14·0–16·0	Up 2·0	—
01·31	10	0·6 i.u. pitressin	12·8–12·6	Down 0·2	—
			12·8–15·0	and up 2·2	—
01·40	11	1·2 i.u. pitressin	12·8–15·4	Up 2·6	—
01·48	12	1·2 i.u. pitressin	12·8–15·0	Up 2·2	Leakage from venous cannula and movement of animal
01·56	13	1·2 i.u. pitressin	12·8–14·4	Up 1·6	—
02·02	14	Unfractionated P.L.P.	12·0–10·0	Down 2·0	—
			12·0–15·6	and up 3·6	—
02·10	15	Unfractionated P.L.P.	10·4– 7·6	Down 2·8	—
			10·4–12·0	and up 1·6	—
02·18	16	0·05 mg. (approx.) histamine acid phosphate	9·5– 5·9	Down 3·6	—
02·20		—	—	—	Died

* Note: 1 c.c. saline injected before every pituitary injection. Except in the case of the first injection these evoked no response.

Conclusions and comments from experiment 1

(1) Response to histamine-free pressor fraction (pitressin) is similar to that of an eutherian mammal (i.e. a simple slow rise). The amount of excitant needed to evoke a given rise is roughly ten times that needed to evoke an equivalent rise in a cat or rabbit similarly anaesthetized.

(2) Comparison of rises to similar doses from a base line of 13 and 14 cm. shows the greater sensitivity of the animal at the former level. Serial doses (9–12) show that *at the arbitary level* chosen there is poor discrimination and no evidence of a strong tolerance developing. Since, of course, a dose-response curve is never straight, it might well be that at another level there would be good discrimination.

(3) Response to histamine-free oxytocic fraction (pitocin) is similar to that of the bird (i.e. a swift depression) except that the depression is more prolonged. The amount of excitant needed is roughly ten times that needed to evoke an equivalent depression in the anaesthetized bird. We have insufficient data to comment on either discrimination between doses of pitocin or the development of tolerance.

(4) There is a depressor response to histamine. Unfortunately our weighed tubes of solid histamine acid phosphate were broken in transit and in the semi-field conditions under which we were working, the dose could only be estimated visually. So the result is only of qualitative value.

(5) The response to mixtures of pitressin and pitocin was a depression followed by a rise. The only two injections (nos. 5 and 7) made with this mixture seemed to show that neither constituent seriously interferes with the effect of the other, both fall and rise being manifested.

(6) The whole question of the unitary or multiple nature of the excitant from the posterior lobe has been reviewed recently by Waring & Landgrebe (1950). In view of the evidence marshalled by them for the close bonding of pressor and oxytocic activities in unfractionated extracts, it is important to know whether the response to unfractionated extract is exactly similar to that elicited by a mixture of the chemically separated activities. For reasons set out in no. 4 above we could not inject carefully measured doses. The injections used (nos. 14 and 15) were of freshly prepared extract from dried glandular material (containing no more than the equivalent of 0·2% histamine acid phosphate) and were, in the absence of a delicate balance, judged visually for quantity. It is evident that the type of response was indistinguishable from that evoked by a mixture of the two separated fractions.

(7) Injection of saline alone had insignificant effects.

(8) In our experience with eutherian mammals, the rise to injections of histamine-free pressor fractions is never preceded by a depression, under properly controlled conditions. If there is a depression it is only transitory and attributable to (*a*) too light anaesthesia, or (*b*) in occasional cases, to preparations very sensitive to the rapid injection of cold saline. In the experiments described here, there was no preliminary fall preceding the rise to pitressin; there was no response to saline save a small and very transitory rise; and there was no histamine injected. Therefore the fall to pitocin and to pitocin-pitressin mixtures must have been due to a pituitary principle.

Conclusions and comments from experiment 2

This preparation exhibited the fall to pitocin and rise to pitressin in a manner similar to the first preparation. It was unmistakably less sensitive to pitressin than the first, and this can probably be attributed to the low base-line (3–4·5 cm.). We have previously found in other animals that below a certain base pressure, the response exhibited is very much less than with a slightly higher pressure; in the rat (Landgrebe, Macauley & Waring, 1946) this threshold is 4 cm. Hg. In the same way, of course, the response is reduced by a high base pressure attained by light anaesthesia or transection of the brain anterior to the medulla. For the same reason

the small depression to pitocin is not surprising and is consistent with our experience with the bird.

Preparation 2. *Male*—22–23 *January* 1949—*working sheet*

Animal etherized; vagi severed; trachea cannulated; spinal cord severed at level of the axis vertebra, and brain destroyed in othodox fashion (see Burn, 1937). Artificial respiration; pump speed 40 strokes per min. No atropine administered.

Time	Ref. no.	injections, etc.	Response	increment or or decrement (cm.)	Notes
18·00	—	Ether	—	—	—
19·55	—	Pithed, artificial respiration	—	—	Pump speed 40/min. cannulae inserted intravenous injections
20·00	—	—	—	—	—
20·15	—	heparin	—	—	—
21·00	1	0·5 i.u. pitressin	3·0–4·2	Up 1·2	—
21·03	2	0·6 i.u. pitocin	4·6–3·6	Down 1·0	—
21·13	3	0·1 c.c. adrenalin (1:100,000)	4·6–5·4	Up 0·8	—
21·16	4	0·2 c.c. adrenalin (1:100,000)	4·6–6·6	Up 2·0	—
21·20	5	1·0 i.u. pitressin	4·6–7·0	Up 2·4	—
21·28	6	1·0 i.u. pitressin	6·2–8·4	Up 2·2	—
21·42	7	1·0 i.u. pitressin	6·2–7·3	Up 1·1	—
21·47	8	1·0 i.u. pitressin	6·2–7·0	Up 0·8	—
32·52	9	1·0 i.u. pitressin	6·2–7·4	Up 1·2	—
22·10	10	1·0 i.u. pitocin	—	—	Carotid cannula slipped so no response recorded
22·14	11	1·0 i.u. pitocin	4·0–3·6	Down 0·4	—
22·16	12	1·0 i.u. pitocin	—	—	No response
22·18	13	1·0 i.u. pitressin	4·0–4·8	Up 0·8	—
22·44	14	1·0 i.u. pitocin	3·0–2·4	Down 0·6	—

Preparation 3. *Female*—23 *January* 1949— *working sheet*

This animal was initially anaesthetized with intraperitoneal Dial supplemented by urethane. The procedure described for preparation 1 was followed and the rise to pitressin and fall to pitocin confirmed. The blood pressure of this animal was consistently 4 cm. Hg and, as was to be expected, neither the rise nor the fall to appropriate stimulation was as great as in the first preparation. We then tried to inhibit the respiratory centre by intravenous injection of soluble pentobarbitone. This was accomplished, the blood pressure fell to 3 cm. and the animal was successfully maintained with artificial respiration, but we were unable subsequently to elicit trustworthy responses with any pituitary preparation.

III. DISCUSSION

With only three animals available we tried to set up the three classes of preparation for which there are comparable data from Eutheria, viz. (*a*) fully anaesthetized with respiratory centre functioning; (*b*) spinal, with artificial respiration; and (*c*) fully anaesthetized with respiratory centre inactivated and artificial respiration. We were very fortunate with respect to (*a*), and sufficient information was obtained from (*b*) to show that in essential respects its behaviour was similar to (*a*). (*c*) was a failure.

We may note in passing the very high blood pressure exhibited by (*a*), but if this has significance it escapes us.

Eutherian mammals treated as either (*a*) or (*b*) exhibit a simple rise in blood pressure to either whole posterior pituitary extract or the pressor fraction. Moderate doses of pitocin* exert no effect measurable by the usual mercury manometer. Injection of enormous doses of pitocin result in a pressor response due to unmasking of the slight pressor contamination.

The chief interest of the present findings is therefore the depressor action of the oxytocic fraction, and the combined depressor-pressor action of whole extract. We have no clue to the seat of action of either, and it therefore remains an open question as to whether both excitants activate the same effectors in opposite ways, or whether their effects are separately localized. In either case the tracings make it clear that the depressor mechanism is more quickly activated that the pressor.

In exploring the phyletic distribution of these two mechanisms we look naturally to the birds and reptiles. A fair amount of information has been published about avian responses, but most work on this subject has been directed towards development of a reliable oxytocic assay, and consequently does not fully answer all the questions we wish to pose. Information about reptilian responses is meagre.

Paton & Watson (1912) first described the depressor response of the decapitate duck to whole pituitary extract. Their tracings show a *simple* precipitate depression. Hogben & Schlapp (1924) confirmed their findings and showed that the effect was evoked by histamine-free extracts and was therefore a pituitary action. Their tracings commonly show a simple fall. When separated fractions became available, Gaddum (1928) showed that the depressor response was attributable to the oxytocic fraction, but he gave no information about the effects of pitressin or of mixtures of pitressin and pitocin.

Coon (1939), Smith (1942), and Smith & Vos (1943) have developed the depressor response of the bird as a biological assay to pitocin, but due to the orientation of their studies they present (to us) a rather confusing picture of the relation of effects evoked by pitocin and pitressin. This much seems clear from their work:

(1) Injections of pitocin evoke a simple fall followed by a very slight rise.

(2) Tolerance is rapidly developed to large doses of pitocin (no fall evoked).

(3) In birds not tolerant to pituitary, pitressin evokes a simple fall and slight secondary rise (greater than pitocin secondary rise).

(4) Pitressin into oxytocic-tolerant fowl evokes 'pure rise' (Coon's words).

(5) Pitocin + pitressin (P: $o < 3{\cdot}5$: 1) evokes a simple fall equivalent to that which would have been evoked by oxytocic content alone.

(6) Pitocin + pitressin (P: $o > 3{\cdot}5$: 1) evokes a fall greater than that attributable to oxytocic content.

These results indicate that the bird response is unique, but work in progress shows that this is not so for all bird preparations. In a fowl heavily anaesthetized with

* With high doses of pitressin, the coronary constrictor action of the drug is shown by transitory heart failure; this may be masked or alleviated if pitocin, which has a dilator effect on coronary arteries, is also present.

phenobarbitone (respiratory centre inhibited) we have obtained a pure rise to pitressin and a fall and subsequent rise to pitocin. Since this rise can be obtained with the first injection, the effect is not to be attributed to pitocin tolerance. We have also obtained a consistent rise to pitressin and fall to pitocin in pigeons heavily anaesthetized with pentobarbitone.

All this needs further analysis, but a simple interpretation and comparison that fits the facts at present is: (*a*) that in the platypus (under the conditions described) and in deeply anaesthetized birds in our hands there is a simple depression to pitocin and a simple rise to pitressin; (*b*) that with a mixture of pitressin and pitocin (and possibly unfractionated extract) the two effects of these excitants follow serially, and while this reaction is simple in the platypus it is complicated in the bird by the double effect of pitressin which first potentiates the depression and later exhibits its own pressor effect in reduced degree.

The scanty information about the effect of posterior lobe extracts on reptiles is as follows: Hogben *et al.* (1924) found that with unfractionated extract Chelonians show a simple slow fall of little magnitude; the present authors (unpublished) have found that in the lizard, *Trachysaurus rugosus*, depression can be elicited by pitocin and a rise by pitressin.

Finally, we may be permitted to speculate about the phylogenetic significance of our findings, irrespective of whether our interpretation of the sequence of events in birds and platypus will stand up to later observation. In the evolutionary dichotomy from reptiles which produced the birds on the one hand, and the eutherian mammals on the other, the avian stem retained both mechanisms (though somewhat complicated), whereas the eutherian stem has lost its depressor mechanism. If we assume that the usual reptilian condition is to have both the pitocin-depressor and the pitressin-pressor mechanism, then in this respect the monotremes show a closer affinity to the sauropsidan stock than to the eutherian. Unfortunately, all therapsids are extinct.

When these observations were completed we heard indirectly that Prof. Matthey of the University of Lausanne had been making investigations on the chromosomes of the platypus, and in response to a request for his general conclusions he courteously wrote us: 'je n'ai que des observations très incomplètes sur les chromosomes de Platypus, mais qui confirment complètement celles de White, sur l'Echidne. Les chromosomes sont de types "Oiseau" ou Tortue. Très nombreux (60–80), distribuès en macro—et en microchromosomes. Si fragmentaires que soient ces observations, elles suffisent pour affirmer que la parente des Monotrèmes avec les Marsupiaux (cf. Gregory, 1940) est exclue totalement. Ils doivent representer un rèmeau independant au voisinage des Oiseaux et des Cheloniens.' Thus our inferences from pharmacological data receive support from an entirely different source.

IV. SUMMARY

1. The carotid blood-pressure response to intravenous injection of histamine-free extracts of mammalian posterior pituitary extracts in the platypus (*Ornithorhynchus*) is described.

2. Pitressin evokes a simple pressor response.

3. Pitocin evokes a simple depressor response.

4. Mixtures of pitressin and pitocin evoke a fall and a subsequent rise.

5. Histamine evokes a depressor response.

6. A preliminary note is made of the simple pressor response to pitressin and depression to pitocin in the deeply-anaesthetized bird and in a lizard.

7. Comparison is made between the responses to posterior pituitary extracts in reptiles, birds, the platypus and eutheria.

8. The possible phylogenetic significance of these observations is discussed.

This work required considerable organization because of the difficulty of obtaining living material. We are heavily indebted to the following individuals: Mr K. W. Taplin for help in converting electrical equipment; Prof. D. Wright for the loan of kymographic apparatus; Mr F. L. Combes and his assistants of the Tasmanian Wild Life Preservation Department; and finally, Prof. V. V. Hickman for generous hospitality at his home and laboratories in Hobart and for his constant support in many directions.

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