in that histamine, tetramethylammonium and an active polypeptide were easily detected; the substances were qualitatively distributed as they had described. Despite repeated and careful extractions of large quantities of anemones (up to 100 g) we have failed to detect 5-hydroxytryptamine (assay limit 10 ng/g), acetylcholine, propionylcholine or butyrylcholine (assay limits 10, 60 and 80 ng/g respectively). A spot resembling tryptamine was noted in many chromatograms, as were spots of high R_F having choline ester-like activity on the guinea-pig ileum; these latter spots have not yet been identified. Noradrenaline, dopamine and dopa were also found (Carlyle, 1969).

The actions of drugs and electrical stimulation were measured on isolated ring preparations of the supra-oral sphincter, suspended from a pair of platinum hooks in sea-water at 15° C. Contractions were elicited by the application through the platinum hooks of square wave pulses of 35 v and 0·1–1·0 msec duration at various frequencies. Contractions were recorded isotonically or isometrically. Both fast and slow contractions were obtained as previously reported by Ross (1957) for preparations from *Calliactus*. Surprisingly, and unexpectedly, responses were often obtained to single shocks in fresh preparations.

The following drugs were without effect on the resting tone, spontaneous activity or responses to electrical stimulation of the isolated preparations; acetylcholine, carbachol, methacholine, eserine, neostigmine, nicotine, atropine, hyoscine, curare, suxamethonium, hemicholinium and choline in concentrations of up to 10^{-2} g/ml.

Noradrenaline, dopamine and isoprenaline in concentrations of up to 5×10^{-3} g/ml. were similarly ineffective, as were a variety of α or β-blocking agents, tryptamine and 3-hydroxytryptamine. Cocaine (10^{-3} g/ml.) caused a prolongation of the response to electrical stimulation. The above results are essentially similar to those reported for *Calliactus* and *Metridium* by Ross (1960), who also found that reserpine was without effect. However in these experiments reserpine (10^{-7}–10^{-6} g/ml. for 12–36 hr) caused changes in the excitability of intact anemones and isolated preparations, as first reported by Pearce (personal communication, 1968). The effects of reserpine may, in many experiments, be reversed by noradrenaline or dopamine. Catecholamines may play some part in neuromuscular activity in the sea anemone.

REFERENCES

APRISON, M. H. (1967). In HOLMSTEDT, BO., Mobilisation of acetylcholine by cholinergic agents. *Ann. N.Y. Acad. Sci.*, **144**, 433–458.

CARLYLE, R. F. (1969). The occurrence of catecholamines in the sea anemone *Actinia equina*. *Br. J. Pharmac.*, **36**, 182P.

MATHIAS, A. P., ROSS, D. M. & SCHACHTER, M. (1960). The distribution of 5-hydroxytryptamine, tetramethyl-ammonium, hormarine and other substances in sea anemones. *J. Physiol., Lond.*, **151**, 296–311.

ROSS, D. M. (1957). Quick and slow contractions of the isolated sphincter of the sea anemone *Calliactus parasitica*. *J. exp. Biol.*, **34**, 11–28.

ROSS, D. M. (1960). The effects of drugs and ions on neuromuscular preparations of sea anemones. II, On sphincter preparation of *Calliactus* and *Metridium*. *J. exp. Biol.*, **37**, 753–774.

Isolation of a new oxytocic peptide from bovine posterior pituitary lobes

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Oxytocin and vasopressin, the polypeptide hormones of the neural lobe of the bovine pituitary gland, can readily be dissociated from the neurophysins and

separated from each other by " Sephadex *G*-25 " chromatography of a protein–hormone complex (Frankland, Hollenberg, Hope & Schacter, 1966). Whereas a rather pure preparation of vasopressin was obtained, the oxytocin was contaminated with less active material. It has been found that re-chromatography of the crude oxytocin on " Sephadex *G*-25 " in the absence of neurophysin resulted in the separation of an oxytocic material, identified as a peptide and which accounted for 3·5% of the weight of the material recovered. Preliminary amino-acid analysis indicates a molecular weight of approximately 3000 for this new oxytocic peptide.

While this peptide possessed an oxytocic activity of 2·8 i.u./mg, as determined on the isolated rat uterus preparation of Holton (1948), the milk ejecting activity assayed in the lactating rat (Bisset, Clark, Haldar, Harris, Lewis & Rocha e Silva, 1967) was more than three-fold greater (9·6 i.u./mg). The uterine stimulating action was not destroyed by incubating the peptide with 0·01 M sodium thioglycollate at 65° for 5 min nor antagonized by $1{\cdot}4\times10^{-6}$M atropine or $3{\cdot}3\times10^{-7}$M phenoxybenzamine. The substance was further characterized by a pressor activity of 0·083 i.u./mg by the method of Dekanski (1952) and an antidiuretic activity of 0·133 i.u./mg as assayed in the rat, under ethanol anaesthesia with constant water load, according to the method of Dicker (1953) with the modifications of Bisset (1962) and Clark & Rocha e Silva (1967). These pharmacological activities, together with its elution volume from a column ($1{\cdot}2\times60$ cm) of " Sephadex *G*-25 " in ammonium acetate buffer pH 5·5 distinguishes the peptide (elution volume 35·8 ml.) from angiotensin (37·4 ml.), acetylcholine (38·2 ml.), oxytocin (41·9 ml.), vasopressin (46·5 ml.) and bradykinin (62·5 ml.). Since there was no significant depletion of adrenal ascorbic acid concentration or rise in plasma corticoid levels in intact rats (Arimura, Saito & Schally, 1967) on the intravenous administration of 6 μg of peptide, it may be concluded that both the ACTH and CRF activities of the material are minimal.

Hawker, North & Zerner (1969) have recently reported the occurrence in the ox hypothalamus of an oxytocic material which may be a peptide. It could be separated from oxytocin by gel filtration on " Sephadex *G*-25 " with an elution volume somewhat less than that of oxytocin: the ratio of the elution volumes being approximately 1·12. This figure is similar to the ratio (1·17) of elution volumes of oxytocin and the peptide obtained from the bovine pituitary posterior lobe in the present work. A further point of similarity between the two oxytocic materials is their resistance to inactivation by thioglycollate.

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REFERENCES

ARIMURA, A., SAITO, I. & SCHALLY, A. V. (1967). Assays for corticotrophin-releasing factor (CRF) using rats treated with morphine, chlorpromazine, dexamethasone and Nembutal. *Endocrinology*, **81**, 235–245.

BISSET, G. W. (1962). Effects of tyrosinase preparations on oxytocin, vasopressin and bradykinin. *Br. J. Pharmac. Chemother.*, **18**, 405–420.

BISSET, G. W., CLARK, B. J., HALDAR, J., HARRIS, M. C., LEWIS, G. P. & ROCHA E SILVA, M. (1967). The assay of milk-ejecting activity in the lactating rat. *Br. J. Pharmac. Chemother.*, **31**, 537–549.

CLARK, B. J. & ROCHA E SILVA, M. (1967). An afferent pathway or the selective release of vasopressin in response to carotid occlusion and haemorrhage in the cat. *J. Physiol., Lond.*, **191**, 529–542.

DEKANSKI, J. (1952). The quantitative assay of vasopressin. *Br. J. Pharmac. Chemother.*, **7**, 567–572.

DICKER, S. E. (1953). A method for the assay of very small amounts of antidiuretic activity with a note on the antidiuretic titre of rat's blood. *J. Physiol., Lond.*, **122**, 149–157.

FRANKLAND, B. T. B., HOLLENBERG, M. D., HOPE, D. B. & SCHACTER, B. A. (1966). Dissociation of oxytocin and vasopressin from their carrier protein by chromatography on Sephadex G-25. *Br. J. Pharmac. Chemother.*, **26**, 502–510.

HAWKER, R. W., NORTH, W. G. & ZERNER, B. (1969). The pharmacology of a new oxytocic principle from ox hypothalamus. *Br. J. Pharmac.*, **35**, 175–186.

HOLTON, P. (1948). A modification of the method of Dale and Laidlaw for standardization of posterior pituitary extract. *Br. J. Pharmac. Chemother.*, **3**, 328–334.

The involvement of histamine in malaria

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An increase in histamine concentration in the blood of *P. knowlesi*-infected rhesus monkeys (*Macaca mulatta*) is primarily concerned, among other vaso-active substances released during the advanced stages of the disease, in the inflammatory " stasis " that often occurs in local vessels. This inflammatory stasis is particularly evident in the brain vessels, and leads to overall disturbances of the blood circulation, which in turn lead to pathophysiological effects such as coma (Maegraith, 1966).

Histamine was extracted from blood of control (non-infected) and infected monkeys by a modification of the method of Barsoum & Gaddum (1935). It was assayed on the guinea-pig isolated ileum suspended in Tyrode solution using histamine acid phosphate (BDH) as a standard.

The histamine extracts were also injected intradermally into guinea-pigs and accumulation of circulating dye (pontamine sky blue, 6BX) at the site of injection was observed as an indication of changes in vascular permeability. The lesions produced by the histamine extracts from five infected monkeys were often more intense in colour than those produced by the same substance extracted from four control monkeys, which, as expected, elicited very weak reactions. The effectiveness of the histamine extracts from the malarial monkey in increasing vascular permeability and causing extensive damage to the endothelial vessel walls was almost completely abolished by pre-treatment of the animals with mepyramine maleate (20 mg/kg), promethazine hydrochloride (20 mg/kg) and diphenhydramine hydrochloride in the same dose.

Diapedesis of leucocytes was observed in 1–2 hr, and cell necrosis was demonstrated in the 24–28 hr old lesions, as evidenced by histological examinations of the skin sections previously fixed in Zenker's fluid.

Histamine has been shown to be one of the factors involved in the widespread breakdown of the blood-brain barrier, since intracranial injections into guinea-pigs (weight 140–200 g) caused exudation of high molecular weight substances such as albumin associated with fluid into the cerebrospinal fluid.

A mean concentration of histamine of 0·15 μg/ml. in the circulating blood was found in *P. knowlesi*-infected monkeys during the late stages of the disease, no histamine was detected in the blood of control monkeys. It is therefore concluded that histamine is one of a group of pharmacologically active inflammatory substances liberated into the circulation when the disease becomes relatively severe and produces a widespread disturbance of the blood-brain barrier. Increased net movement of heavy protein molecules such as albumin across the relevant membranes would suggest the occurrence of local " stasis " in the small vessels of the brain substance