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A Histamine-Like Component of Commercial Pitressin.* (22219)

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Several investigators have assigned an important role to pitressin as a releasing agent for ACTH. Thus, Mirsky, Stein and Paulisch(1), suggested that the antidiuretic hormone of the posterior pituitary (ADH, Vasopressin) may serve as a mechanism for ACTH release under stress. McCann and Brobeck (2), also implicated ADH as a factor for release of corticotropin. They destroyed the supraoptico-hypophyseal tract thereby inhibiting the usual output of ACTH in response to stress. However, when animals bearing hypothalamic lesions were injected with commercial pitressin, ACTH was released. Guillemin and Hearn(3), employing rat pituitaries and a tissue culture technic, demonstrated that pitressin releases ACTH from the adenohypophysis *in vitro,* whereas highly purified arginine-vasopressin obtained from du Vigneaud was without effect. The ACTH releasing activity of pitressin was attributed to an unknown contaminant probably originating in the hypothalamus. Saffran, Schally and Benfey(4), also reported that vasopressin contained a corticotropin-releasing factor as an impurity which they were able to separate from vasopressin by paper chromatography.

In view of reports(5-7) that extracts prepared from posterior pituitary powder and fresh glands contain histamine, it was considered possible that minute amounts of the amine bound to the polypeptide were carried through the extraction procedure for commercial pitressin and persisted in the finished product. To anticipate, it can be stated that commercial pitressin contains a component of unknown nature which exhibits some of the physiological characteristics of histamine and which for brevity we shall hereafter refer to as "histamine-like."

Methods. Pitressin† (Vasopressin Injection, 20 units/cc, Parke, Davis and Co.) was extracted with strong acid at boiling temperature. The material was pooled in batches containing 3200 units, in approximately 160 cc, and sufficient concentrated HCl added to make an 18% solution. The fluid was divided among 3 flasks equipped with water cooled reflux condensers and the pitressin boiled vigorously for 1 hour. The commercial material is preserved with 0.5% chlorobutanol which readily crystallizes on the condenser and was removed. After 1 hour the water condensers were replaced by reflux air con-

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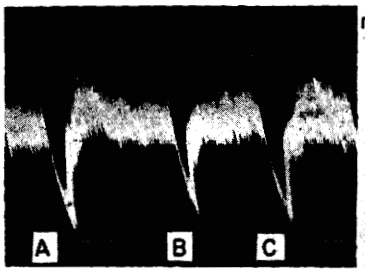

Fig. 1

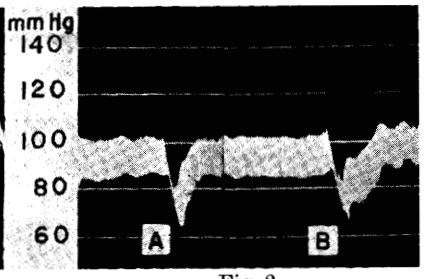

Fig. 2

FIG. 1. Cat, 2.3 kg, atropinized, etherized, carotid blood pressure. A: Pitressin extract 2 cc i.v. (625 units/kg). B: Histamine base 1.81 γ/kg i.v. C: Histamine base 3.62 γ/kg i.v. One cc of pitressin extract is equivalent to 4.17 γ/cc histamine base.

FIG. 2. Cat, 1.9 kg, atropinized, etherized, carotid blood pressure. A: Histamine base 0.3 γ/kg i.v. B: Oxytocin extract 1.5 cc i.v. (625 units/kg). Slight depressor effect. One cc of oxytocin extract is equivalent to 0.38 γ/cc histamine base compared to pitressin extract = 4.17 γ/cc of histamine base.

densers and the boiling continued for an additional 30 minutes or until the fluid was reduced to 10-15 cc. The contents of the flasks were then combined and the extract taken to dryness under reduced pressure at 40°C. The residue was washed 3 times with absolute ethanol which was removed *in vacuo*. Water was added to the desired concentration and the pH of the solution raised to that of commercial pitressin, *i.e.*, pH 3. A fine precipitate forms which is removed by centrifugation. Crude extracts prepared in the manner described retained no trace of pressor activity when tested on the blood pressure of the dog and cat. The histamine-like substance in the extracts was tested by its effect on smooth muscle of the guinea pig ileum and blood pressure of the etherized, atropinized cat. Segments of the lower ileum of fasted young guinea pigs were suspended in a 10 cc chamber containing warm, oxygenated, magnesium-free Tyrode's solution to which atropine had been added in the concentration 0.01 γ/cc. The active agent in all extracts was determined as quantitatively equivalent to histamine base and pyribenzamine was employed for inhibiting contractions of the ileum and for preventing the decline in blood pressure of the cat following extract or histamine administration.

Results. The pressor action of pitressin is abolished by boiling for 30-40 minutes in 1% HCl but a pronounced histamine-like effect is exhibited by concentrated extracts only after prolonged boiling in much stronger acid. Fig. 1 shows the effect of a concentrated extract representing 762.5 pitressin units/cc, or a 38-fold concentrate of acid-boiled commercial material. Strong depressor activity occurred which resulted in a 40 mm Hg fall in blood pressure when 2 cc were given by vein to the etherized, atropinized cat. The extract was estimated to contain activity equivalent to 4.17 γ/cc of histamine base. Oxytocin, prepared in the same manner as pitressin extract, concentrated to the same degree and administered in the same dosage to the atropinized, etherized cat, gave minor depressor effects (Fig. 2). The pitocin contained activity approximating 0.38 γ/cc of histamine base, hence possessed less than one-tenth the activity of pitressin. Apparently the histamine-like substance in the posterior lobe is chiefly confined to the pitressin fraction.

Concentrated pitressin extracts also induced typical histamine-like responses when tested on the guinea pig ileum (Fig. 3). The extracts contained 125 original pitressin units/cc, and the histamine base equivalent was approximately 0.3 γ/cc. Pyribenzamine abolished the gut response to both extract and histamine (Fig. 3 C, D).

McCann and Brobeck(2), were unable to deplete the adrenal ascorbic acid of hypophysectomized rats by intravenous injection of pitressin. We also obtained negative results in similar experiments. Extract concentrates representing 803 units/cc, and containing significant amounts of histamine-like activity in tests on the cat's blood pressure (35 mm Hg

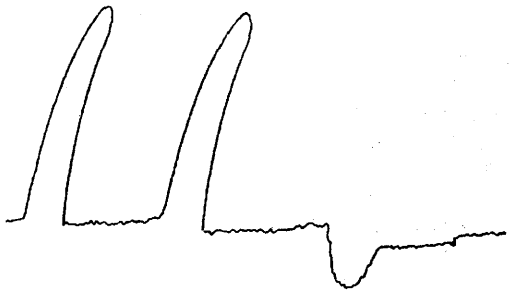

FIG. 3. Isolated ileum of guinea pig. Doses given per cc of bath fluid. A: Histamine base 0.015 γ/cc. B: Pitressin extract 0.50 cc. One cc of extract equivalent to 0.3 γ histamine base. Between B and C pyribenzamine given 0.5 γ/cc. C: Pitressin extract 0.5 cc. D: Histamine base 0.015 γ/cc.

fall), failed to produce a decline in adrenal ascorbic acid of 8 hypophysectomized rats. The extract was injected in 0.4 cc doses by vein. We are indebted to Dr. H. Megel of the Princeton Laboratories, Inc., for making the Sayers test for us.‡

Discussion. Abel and Kubota(5) reported histamine present in posterior pituitary powders and extracts. Subsequent investigators failed to detect the amine in either powders (8) or fresh glands(9). Recently, Harris *et al.*(7) were able to extract histamine from brain, hypothalamus, median eminence and both anterior and posterior pituitary tissue. The present writers have found a histamine-like substance present in extracts of acetone dried powders of the posterior lobe. Four grams of such powder when subjected to the complete extraction procedure of Code and Ing(10) for determining the presence of histamine in blood plasma and cells, and highly concentrated, contained activity approximating that of 4.19 γ/cc, of histamine base when assayed against the standard and using the guinea pig ileum and blood pressure of the cat as test objects.

‡ Recent experiments show the extract to be active in releasing ACTH from halved anterior pituitaries incubated in the Warburg apparatus using the Saffran *et al.* technic. Minute amounts of the incubation fluid given i. v. to 24 hypophysectomized rats induced a significant fall in adrenal ascorbic acid of 120 mg % in the Sayers test. Incubation fluid from the control half of the same glands gave negative results.

We were not successful in removing the active agent from pitressin or acetone dried powder by aqueous or dilute HCl extraction. It required strong acid and 90 minutes of boiling temperature to accomplish its removal. Apparently the histamine-like component of pitressin is bound to the pressor polypeptide and is released, or at any rate rendered capable of inducing physiological reactions characteristic of histamine, by some degree of acid hydrolysis. Pyribenzamine readily inhibited the response of the gut to extract and to pure histamine dihydrochloride, but it did not entirely eliminate the depressor effect of 2 cc of concentrated extract on the blood pressure of the atropinized cat. Frandsen(11) reported that the active histamine-like substance found in ultrafiltrates of dog serum differed from pure histamine since it is not destroyed by added histaminase nor attacked by histaminase of serum. The exact nature of the histamine-like component in pitressin is unknown but if it is histamine it does not appear to be present as the free amine. The writers tentatively regard it as probably histamine combined with an amino acid or peptide, but pending its isolation in pure form this is merely speculation. Certain facts seem to favor the idea and may be summarized as follows: In tests on the guinea pig ileum the behavior of the active substance in the extracts cannot be distinguished from that of histamine and like the amine its action is abolished by pyribenzamine. When highly concentrated it exerts strong depressor effects on blood pressure of atropinized, etherized dogs and cats and the slope of the pressure falls and subsequent recovery is identical with that elicited by histamine. The effect of the extract upon ileum and blood pressure can be quantitated using histamine base as the standard. Similar extracts of oxytocin which differs from pitressin by two amino acids, elicit small depressor responses in atropinized cats, but they are less than one-tenth the magnitude of those induced by pitressin. Dosage in both cases was 625 units/kg. Extracts of 4 crystalline proteins and of each of the amino acids in vasopressin also gave negative results. Investigators(2-4) have shown

that oxytocin, unlike pitressin, does not release ACTH. Histamine as the dipicrate has been recovered from posterior lobe tissue (5,6) and a histamine-like substance has been reported present in this gland and also in neighboring hypothalamic structures(7). None of these data furnish conclusive evidence that a histamine complex of some sort is the active component of pitressin extracts but they are suggestive, and it seems not unreasonable to make such an assumption as a working hypothesis.

Summary. Commercial pitressin contains minute amounts of a histamine-like component apparently bound to the polypeptide but which can be concentrated and rendered capable of inducing physiological responses characteristic of histamine when pitressin is subjected to strong acid hydrolysis and the pressor activity destroyed. Extracts of commercial oxytocin contained but one-tenth the vasodepressor activity of pitressin extracts.

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Effects of Glucagon on Plasma Potassium.* (22220)

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Glucagon(1) has a selective action on liver in which the activity of phosphorylase is increased and this action may be the basis for increased hepatic glycogenolysis and hyperglycemia(2). The effects of glucagon mimic epinephrine action only in the liver. Glucagon has no glycogenolytic action on muscle, no cardiovascular effect(3), and no effect on hepatic vessels(4). These properties make glucagon a useful pharmacologic tool for studying the accompaniments of hepatic glycogenolysis. Our aim was to determine whether hepatic glycogenolysis was necessarily associated with increased blood potassium.

Although epinephrine hyperkalemia has been related to hepatic glycogenolysis, several facts appear inconsistent with this simple relationship. Subcutaneous epinephrine produces hyperglycemia without an associated hyperkalemia. Intravenous epinephrine produces hyperkalemia which persists for only 3 to 5 minutes(5) and hyperglycemia which continues for one-half to one hour. The relative potencies of sympathomimetic amines for the hyperkalemic effect are markedly different from their relative potencies for hyperglycemia(6,7). Dibenamine is effective in suppressing epinephrine hyperkalemia(7), but it is extremely weak as an antagonist to epinephrine hyperglycemia(8). Epinephrine hyperkalemia occurs in animals treated with phloridzin in order to deplete liver glycogen (9). Constrictor agents with weak hyperglycemic activity produce hyperkalemia(5, 10). These discrepancies were the basis for

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