PITUITARY HORMONE RELEASING OR INHIBITING ACTIVITY OF METAL IONS PRESENT IN HYPOTHALAMIC EXTRACTS[1]

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SUMMARY

In vitro trophin-releasing and inhibiting activities of certain purified fractions from bovine hypothalamus were apparently due primarily to the presence of divalent metals. The most abundant metal was copper which at concentrations less than 1 μg/ml markedly stimulated the release of all hormones. Zinc ion which was much less potent than copper, inhibited release of prolactin but stimulated release of the other hormones. Nickel, as low as 1 μg/ml, specifically inhibited prolcatin release; at much higher levels it enhanced release of the other hormones.

Several fractions purified from bovine hypothalamic tissue were found to retain their "releasing factor" activity after destruction of organic components. Analysis for inorganic constituents indicated that our purification procedures had resolved individual divalent metal ions which appeared to be responsible for the releasing activity of their respective fractions. These metal-containing fractions could be distinguished from those containing active organic, presumably peptide, components.

Besides calcium and magnesium, the cations in greatest abundance were copper, zinc and nickel. Fractions containing zinc and nickel usually showed prolactin (PRL)-inhibiting activity, with or without concomitant release of other hormones. Fractions containing copper plus nickel and/or zinc promoted release of all hormones, except for PRL whose release was diminished below control level or was unaffected.

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EXPERIMENTAL

Bovine pituitary glands from animals of unspecified age and sex were obtained at a slaughterhouse about 30-60 min after death of the animals. The glands were maintained at 25°C during transport to the laboratory and during all manipulations prior to incubation at 37°C. For each experiment, 10-15 anterior lobes were sliced with a razor blade and diced into 1 mm cubes with a mechanical chopper. Homogeneous 300 mg portions of the diced tissue were added to 25 ml Erlenmeyer flasks containing 5 ml Krebs-Ringer-bicarbonate (with 200 mg% glucose), pH 7.4, and incubations carried out in a Dubnoff shaker at 37°C under 95% O_2/5% CO_2. The tissue portions were individually pre-incubated for 1 hour with the medium renewed every 20 min. The medium from preincubation was discarded, 5 ml fresh medium added, test substances added in 0.1 ml of buffer, and flasks were incubated for 2 hours. The medium was then decanted, centrifuged to remove particulate material, and frozen.

Each experiment consisted of 18 treatments (several test substances at various concentrations and a control), each duplicated. The treatments were assigned to the flasks in a randomized block pattern, each block consisting of 18 tissue aliquots weighed and added to the flasks consecutively over a short interval of time. The incubation media were assayed for the bovine hormones by radioimmunoassay. The solid phase procedure described by Catt et al., (1968) was used for PRL, GH, TSH and LH, and the charcoal-dextran method of Donald (1968) for ACTH. FSH was not determined in these studies due to technical difficulties in the radioimmunoassay. The values for hormone levels in the media were log-transformed to eliminate variance heterogeneity. Subsequent statistical analysis consisted of a randomized block analysis of variance which incorporated Tukey's test for non-additivity as an outlier screen (Steel and Torrie, 1960). The treatment means were compared with the control by Duncan's new multiple-range test (Steel and Torrie, (1960).

We have previously described additional details of the in vitro incubation

system and of statistical and technical aspects of the radioimmunoassays (LaBella and Vivian, 1971; Vivian and LaBella, 1971a, 1971b). Commercial chloride salts of Cu^{++}, Zn^{++}, and Ni^{++} were used for these studies. Stock solutions of these salts were analyzed for the presence of other metals by atomic absorption; no significant contamination by other metal ions was detected in any of the solutions. Aliquots of 0.1 to 0.2 ml of the metal salt solutions were added to each flask. Control experiments demonstrated that the metals did not interfere with the hormone radioimmunoassays.

RESULTS AND DISCUSSION

The metal-induced augmentation of release of GH, TSH, LH, and ACTH, shows a decreasing potency in the order $Cu^{++} > Zn^{++} > Ni^{++}$; the dose-response curves for all four hormones are very similar (Figure 1). At the highest concentrations of Cu^{++} there appears to be a falling-off of the response, this bell-shaped curve being a general characteristic of dose-response curves. The response of the lactotrope, i.e. the PRL-producing cell, to the three metal ions was quite different from that of the other cell types.

Cu^{++} caused a 10 to 15 fold increase in the release of GH, TSH, LH and ACTH, in contrast to only a doubling of PRL release. Whereas the descending limb of the dose-response curve for Cu^{++} on release of the five hormones is just apparent at the higher levels of the metal, inhibition below control level is seen for PRL release. Furthermore, Zn^{++} or Ni^{++}, 6×10^{-5}M, caused a significant inhibition of PRL release which was intensified as the concentration of either metal is increased. Over the range of concentrations of Ni^{++} and Zn^{++} tested, significant increases in release occurred for GH, TSH, LH, FSH and ACTH at the two highest levels only. The only effect of these two metals on PRL release is one of inhibition, and this occurs at relatively low concentrations.

The lactotrope has previously been reported to exhibit unique responses to certain manipulations in vitro. For example, increasing the

potassium ion concentration to several times the normal level results in a marked rate of release of each of the anterior pituitary hormones, with the exception of PRL whose release is little affected (Parsons, 1970; MacLeod and Fontham, 1970). Also, we have demonstrated an anomalous response of the PRL cell to brief exposure to cold (LaBella et al., in press). The unique properties described probably reside in the structure of the plasma membrane, and are further indicated in the present study by the atypical response of the lactotrope to divalent metals. Reynolds (1972) has reviewed the evidence that inorganic cations are essential for the stability of biological membranes; a basis for the difference between the responses of the lactotrope and other pituitary cell types may reside in her proposal that differences in minor lipid constituents could be responsible for varying types of lipid-protein-metal complexes in membranes. Qualitative differences in the minor components could, presumably constitute a basis for functional differences among cell membranes.

The exact mechanism(s) whereby these divalent cations enhance or inhibit pituitary hormone release remains to be elucidated. Several loci of action are probably affected. It is of interest that copper and zinc increase mitochondrial permeability in vitro in dose-response relationships, relative to one another, very similar to those reported here for hormone release (Hwang et al., 1971). Nickel has been reported to support contraction in frog skeletal muscle by releasing bound calcium (Frank, 1962) and to uncouple excitation from contraction in mammalian cardiac muscle by competing with Ca for the contractile mechanism (Ong and Bailey, in press). Zinc and copper were found to be the most potent of the divalent metals in inhibiting brain ATPase in vitro and in vivo (Donaldson et al., 1971).

Whether or not essential trace metal ions in the central nervous system play a physiological role in the control of anterior pituitary secretion, certain observations may be relevant. Among these is the fact that the brain is rich in metalloproteins (Scheinberg and Sternlieb, 1960; Crawford and

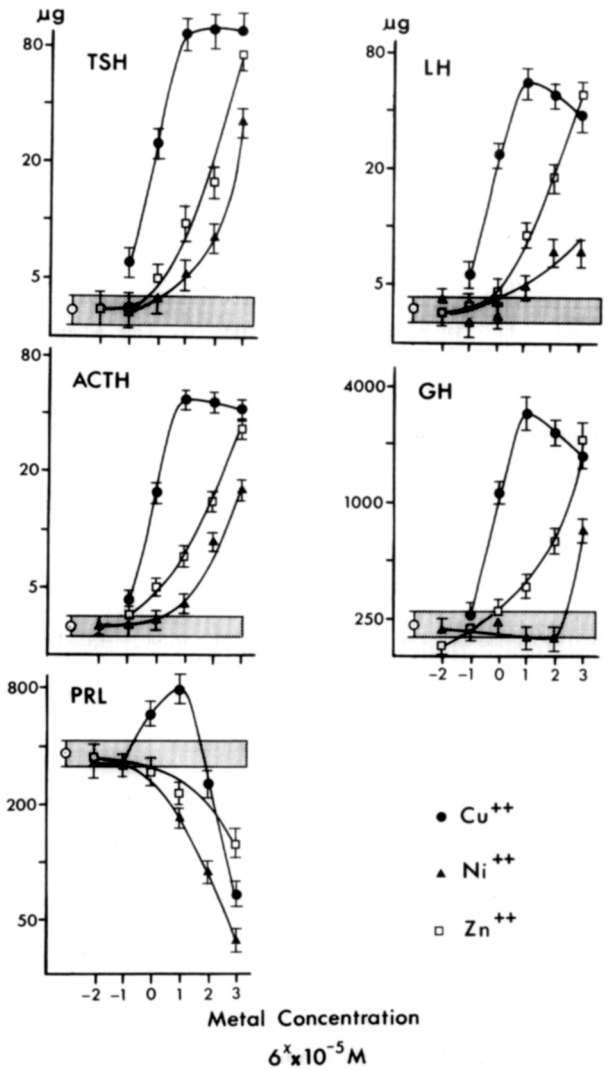

FIGURE 1. Dose-response curves for release of pituitary hormones in vitro in response to metal ions. All hormones were assayed in each incubation flask. The means and standard errors are derived from 4 replicates. The shaded area represents the mean and S.E. of control flasks.

Connor, 1972; Nielsen, 1971); furthermore, certain metal salts apparently can release pituitary hormones in vivo, since copper salts have been reported to induce ovulation in animals upon intravenous or intrahypophysial injections (Friedman, 1941). Also, amino acid and/or peptide complexes with metals, known to be present in tissue extracts, may be physiological modulators of brain and pituitary. (The metal ions which we have isolated from hypothalamic

tissue appear to be, at least in part, complexed with amino acids and/or peptides). It is quite clear that at least some hypothalamic "releasing factors" are peptides which have been identified and synthesized. However, our findings do point out that hormone-releasing and other activities in tissue extracts may be due, at least in part, to the presence of inorganic constituents.

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