Isolated Pituitary Cells: CRF-Like Activity of Neurohypophysial and Related Polypeptides[1] (37386)

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The role of vasopressin in the regulation of corticotropin (ACTH) secretion is enigmatic: in spite of repeated observations that vasopressin is an effective stimulus to ACTH release (2–5) there is much evidence to indicate that it is chemically distinct from the psysiologically important hypothalamic mediator of ACTH secretion, corticotropin releasing factor (CRF) [see (6, 7)]. We now present studies which, we believe, provide some insight into this problem: the CRF-like activity of vasopressin and related polypeptides is distinguished from that of hypothalamic median eminence (HME) extract by their bioassay (log dose response) characteristics and evidence is presented that these polypeptides are partial agonists of ACTH secretion.

Materials and Methods. CRF bioassays were conducted by determining the rate of secretion of ACTH from isolated pituitary cells incubated in the presence of graded doses of test substance (polypeptide or HME extract). The techniques employed for the preparation and incubation of these cells have been described in detail (8, 9). In brief, pituitary cells were dispersed from the anterior lobes of male Sprague–Dawley rats by a combination of trypsin and mechanical agitation; intact animals, or in some cases, as indicated, animals adrenalectomized 2 wk previously and maintained on 0.9% saline drinking solution were used as donors of pituitary tissue. The cells were collected by centrifugation and resuspended to a concentration of 1 pituitary equiv/ml in Krebs–Ringer bicarbonate buffer containing glucose (0.2%), bovine serum albumin (0.5%), and lima bean trypsin inhibitor (0.2%). Aliquots of cell suspensions were incubated for 40 min in the presence of vehicle (controls) or various doses of test substance (polypeptide or HME extract); the cells were removed by centrifugation and the separated medium was acidified and appropriately diluted for ACTH bioassay as described by Sayers, Swallow and Giordano (10).

Acid extracts of rat HME were prepared as previously described (9). Natural arg-vasopressin was extracted from bovine neurohypophyses (11) and had a pressor activity of 320 mU/μg (12). Synthetic arg-vasopressin, lys-vasopressin, 8-homolys-vasopressin, 1-deamino-8-homolys-vasopressin, oxytocin, and pro-arg-gly-NH_2 were kindly provided by Dr. M. Bodanszky, Case Western Reserve University, Cleveland, OH; synthetic arg-vasotocin, [*N*-α-triglycly-8-lys] vasopressin, [1-*N*-carbamoyl-hemicystine-2-*o*-methyltyrosine]-oxytocin, and pro-leu-gly-NH_2 were kindly provided by Dr. J. Rudinger, Institute for Molecular Biology and Biophysics, Swiss Federal Institute of Technology, Zurich, Switzerland; and, synthetic $ACTH^{4-10}$ was kindly provided by Dr. W. Rittel and Dr. P. A. Desaulles, Ciba-Geigy AG, Basel, Switzerland.

Results. Isolated pituitary cells, prepared from the adenohypophyses of intact rats, secrete ACTH in response to vasopressin and related polypeptides (Table I). The active, *i.e.*, CRF-like peptides include natural and synthetic arg-vasopressin and also synthetic lys-vasopressin, 8-homolys-vasopressin, 1-deamino-8-homolys-vasopressin, oxytocin and arg-vasotocin. There is no discernible difference in the CRF-like potencies of the peptides; in each case the minimum effective dose (MED) is approximately 0.05 to 0.1 μg. Similarly, the maximum responses observed are approximately the

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TABLE I. Secretion of ACTH by Isolated Pituitary Cells Prepared from Intact Rats in Response to Neurohypophysial and Related Polypeptides.

Compound	Dose (μg)	ACTH, % control[a] (mean ± SEM)	No. of observations
Arg-vasopressin	0.0025	108	2
	0.025	141	2
	0.125	147 ± 16	5
	0.25	154 ± 8	29
	1.0	160	2
	10.0	158 ± 18	6
Lys-vasopressin	0.05	138	2
	0.10	135	2
	0.25	143	2
	1.0	148	2
8-Homolys-vasopressin	0.05	161	2
	0.10	155	2
	1.0	158	2
1-Deamino-8-homolys-vasopressin	0.05	155	2
	0.50	162	2
	1.0	171	2
	10.0	142	2
Oxytocin	0.20	132	1
	1.0	146	2
	10.0	147	2
Arg-vasotocin	0.01	98	2
	0.10	144	2
	0.20	159 ± 12	10
	0.45	133 ± 6	5
	1.0	160 ± 10	11
	2.5	176 ± 10	6
	10.0	150	2

[a] ACTH secretion in the presence of polypeptide is expressed as the percentage of control secretion (vehicle only) determined in the same experiment.

same and in all cases less than 200% of control. Several other peptides tested for CRF-like activity, were found to be inactive. These inactive compounds and doses tested include: [*N*-α-triglycyl-8-lys]-vasopressin, 2.5 μg; the *N*-terminal tripeptide of oxytocin, pro-leu-gly-NH_2, 2.5 μg; the *N*-terminal tripeptide of arg-vasopressin, pro-arg-gly-NH_2, 1 and 10 μg; and, the heptapeptide fragment of ACTH, $ACTH^{4-10}$, 2 and 200 μg. In one experiment, [1-*N*-carbamoyl-hemicystine-2-*o*-methyltyrosine]-oxytocin, was observed to elicit significant ACTH secretion at a dose of 5 μg; however, in three other experiments it was found inactive at doses of 2.5, 10, and 20 μg.

Figure 1 demonstrates the response of isolated pituitary cells, prepared from the adenohypophyses of adrenalectomized rats, to graded doses of arg-vasopressin, arg-vasotocin and HME extract. Both peptides elicit significant secretion of ACTH. However, there are striking differences in the maximum responses observed for peptide vs HME extract.

Figure 2 displays the results of experiments in which isolated pituitary cells from intact rats were incubated in the presence of various doses of HME extract plus or minus 10 μg arg-vasopressin. The rate of ACTH secretion in the presence of HME plus arg-vasopressin (A_{HV}), minus the rate of ACTH secretion in the presence of HME only (A_H), *i.e.*, ($A_{HV} - A_H$), is plotted as

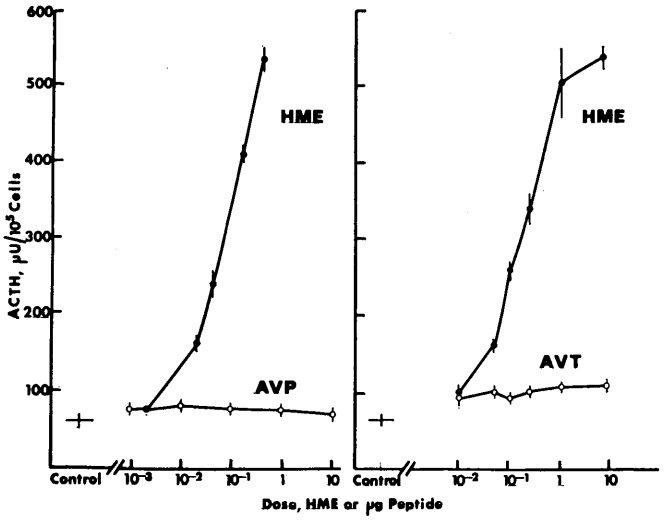

FIG. 1. ACTH secretion vs log dose of HME extract or neurohypophysial peptide. Isolated pituitary cells were prepared from 14-day adrenalectomized rats according to procedures previously described (8, 9); the cells were preincubated for 10 min; vehicle (controls), HME extract, or neurohypophysial peptide [arg-vasopressin (AVP), left panel, arg-vasotocin (AVT), right panel] was added and the incubation continued for an additional 40 min; doses are given as μg peptide or fraction of a rat HME; vertical lines indicate standard error of ACTH bioassay.

a function of A_H observed at each dose of HME. At low rates of ACTH secretion, $A_H < 80\ \mu U/10^5$ cells, and hence at low doses of HME, the quantity $(A_{HV} - A_H)$ is positive, *i.e.*, ACTH secretion in the presence of HME plus vasopressin is greater than in the presence of HME only. However, there is a significant negative linear regression of $(A_{HV} - A_H)$ on A_H (correlation coefficient $r = -0.746$, $p < 0.01$) such that at $A_H > 80\ \mu U/10^5$ cells, the quantity $(A_{HV} - A_H)$ becomes negative, *i.e.*, ACTH secretion due to a given dose of HME plus 10 μg arg-vasopressin is less than that due to the given dose of HME only.

Discussion. The ability of the naturally occurring neurohypophysial hormones to alter the secretion of ACTH is well documented. Arg- and lys-vasopressin are consistently observed to stimulate ACTH secretion both *in vivo* (3, 5) and *in vitro* (2, 4). Oxytocin is devoid of CRF activity in the hypothalamic lesioned rat (13), but exhibits CRF activity, albeit at low potency, in the pentobarbital, morphine "blocked" rat (3) and also in the *in vitro* pituitary fragment assay (2). Arg-vasotocin does not release ACTH when injected into the third ventricle of dogs (14), but is active as a CRF in the pituitary fragment assay (15). A variety of synthetic analogues of the neurohypophysial hormones also exhibit CRF activity. Doepfner, Stürmer and Berde (3) and Martini (16) reported a good correlation between the CRF and pressor activity of natural neurohypophysial peptides but little correlation between these activities for a series of analogues. Arimura, Schally and Bowers (17) also noted that the CRF activity of lys-vasopressin and two of its analogues was not related to their pressor or antidiuretic activities. In the present study, the naturally occurring hormones, arg-vasopressin, lys-vasopressin, oxytocin and arg-vasotocin, as well as the synthetic analogues, 8-homolys-vasopressin and 1-deamino-8-homolys-vasopressin, the latter compound being a more potent pressor and antidiuretic agent than arg-vasopressin (18), all exhibited approximately equivalent CRF-like activity, both in terms of MED and maximum ACTH secretion observed (Table I), with no relation to their pressor activities. As we have previously reported, the CRF-like activity of arg-vasopressin requires calcium ion (19), is inhibited by physiologi-

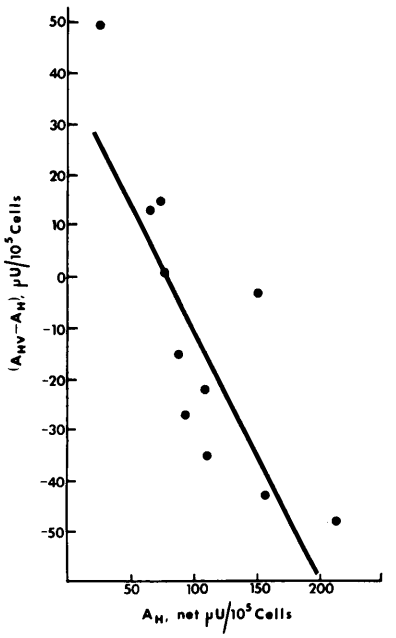

FIG. 2. The effect of arg-vasopressin (AVP) on HME induced ACTH secretion. Isolated pituitary cells from intact rats were preincubated for 10 min; vehicle was added to controls and a given dose of HME only, or that dose of HME plus 10 μg AVP to experimentals, and the incubation continued for 40 min. The data points, representing the differential ACTH secretion by incubates receiving HME plus vasopressin (A_{HV}) and incubates receiving HME only (A_H), *i.e.*, ($A_{HV} - A_H$), are plotted as a function of A_H.

cal concentrations of corticosterone (19), and is energy dependent (9). These findings suggest that the secretion of ACTH in response to the polypeptides is not simply due to a "nonspecific" interaction. Furthermore, several other polypeptides did not elicit significant ACTH secretion from isolated pituitary cells at the doses tested. The inactive compounds included: [*N*-α-triglycyl-8-lys]-vasopressin, a compound with pronounced, long-lasting pressor and antidiuretic activity, which has previously been reported to be devoid of CRF activity in man (20); the *N*-terminal tripeptide of oxytocin, pro-leu-gly-NH_2, reported to be involved in the regulation of MSH secretion (21); the *N*-terminal tripeptide of arg-vasopressin, pro-arg-gly-NH_2; [1-*N*-carbamoyl-hemicystine-2-*o*-methyltyrosine]-oxytocin, reported to be an antagonist to many of the actions of oxytocin and vasopressin (22); and, the heptapeptide fragment of ACTH, $ACTH^{4-10}$. This latter compound exhibits CRF activity in the *in vitro* pituitary fragment assay (23) but is devoid of CRF activity *in vivo* (24).

Our findings are thus similar to earlier reports which indicate that the neurohypophysial polypeptides exhibit CRF activity and that this activity is retained in spite of rather extensive molecular alterations. The new and important finding revealed by the isolated pituitary cell system is that the CRF-like activity of these polypeptides can be distinguished from that of HME-CRF in terms of their bioassay (log dose response) characteristics. Specifically, the polypeptides induce a maximum rate of ACTH secretion significantly less than that induced by HME-CRF. Isolated pituitary cells prepared from the adenohypophyses of intact rats display maximum ACTH secretion of less than 200% control in response to all the polypeptides tested (Table I); in contrast we have consistently observed that HME extract elicits significantly greater ACTH secretion (0.2 HME, 359 ± 29% control, mean ± SEM, $n = 14$). When the log dose–response curves for neurohypophysial polypeptides and for HME extract are compared using cells prepared from the glands of adrenalectomized rats (Fig. 1) the contrast in maximum response is even more apparent. On the other hand, previously reported assay data have been interpreted to mean that neurohypophysial polypeptides and HME extract are identical in their biological behavior. In *in vivo* assays, Arimura, Schally and Bowers (17) found the slopes of log dose–response curves for lys-vasopressin and two of its analogues to be not significantly different from that for a porcine HME-CRF preparation. In *in vitro* assays using the pituitary fragment technique, Saffran (2) noted the similarity of the slopes of log dose–response curves to neurohypophysial peptides and to a crude "CRF" preparation. More recently this same laboratory (5), using both the *in vitro* pituitary fragment technique and an *in vivo* median eminence lesioned preparation, concluded that

"there was no real difference in the response to vasopressin and median eminence CRF."

The relatively low rate of release of ACTH from isolated pituitary cells in response to neurohypophysial and related polypeptides may of course be due to an artifact of the isolated cell technique. For example, it might be argued that the procedures employed for the preparation of the cells damage or destroy "receptors" for the polypeptides. Although this possibility is not readily amenable to study, it should be noted that if this is the case, the destructive procedures are apparently rather selective for receptors to these polypeptides since ACTH release in response to HME extract appears quite normal. Using cells from intact rats, we have observed responses of greater than 200 μU ACTH/10^5 cells/40 min (unpublished data). Based on estimates that the rat adenohypophysis contains approximately 3×10^6 cells (25), this corresponds to about 10 mU of hormone/pituitary/hr, a value which is comparable to the maximum release of ACTH from the intact gland *in situ*. On the other hand, the relatively high release of ACTH in response to neurohypophysial and related polypeptides observed in previous assays, may be artifacts of these techniques. *In vivo* assays are rendered suspect because of the uncertainty that the polypeptide is acting directly on the adenohypophysis. In addition to its CRF-like activity, vasopressin in appropriate doses, has been observed to exert a direct steroidogenic influence on the adrenal cortex (26), potentiate the action of CRF at the level of the adenohypophysis (27), and release endogenous CRF at the level of the hypothalamus (28). In *in vitro* assays employing pituitary fragments, because of the long incubations employed [up to 4 hr (4)] and relatively large size of the fragments, it is possible that cells in the interior of the tissue become necrotic and undergo drastic permeability changes. For example, Fleischer and Vale (4) observed that vasopressin in effective doses consistently released ACTH from pituitary fragments only if they were preincubated for 3 hr. They reasoned that "something is happening to the pituitary tissue that either makes this tissue more accessible or more responsive to vasopressin." Similarly, DeWied *et al.* (29) reported the arg-vasopressin did not augment the secretion of ACTH from pituitary fragments which had been preincubated for only 20 min.

The findings in the isolated pituitary cells indicate that the polypeptides and HME extract release ACTH via different cellular mechanisms or, alternatively, that the polypeptides operate through a common mechanism with HME extract but with reduced "efficacy" in the manner of a partial agonist (30). In experiments designed to test these alternatives, we determined the rate of secretion of ACTH from isolated pituitary cells incubated in the presence of various doses of HME extract plus or minus 10 μg of arg-vasopressin. As may be seen from Fig. 2, at appropriate dose ratios, arg-vasopressin inhibits HME induced ACTH secretion. This finding, coupled with the observation that the maximum rate of ACTH secretion induced by neurohypophysial and related polypeptides is less than that induced by HME extract, is compatible with the view that the polypeptides are partial agonists of ACTH secretion. If this is the case it might be expected that the polypeptides bear a structural resemblance to HME-CRF, so that they are able to activate the ACTH secretion mechanism, but unable to do so with full efficacy. In this regard, the recent report of Saffran *et al.* (31) that the ring portion of vasopressin, pressinoic acid, is a more potent CRF than lys-vasopressin itself is of interest, perhaps indicating that this compound is structurally more similar to CRF than the intact neurohypophysial hormone.

Summary. Neurohypophysial and structurally related compounds elicit secretion of ACTH from suspensions of isolated pituitary cells. However, the maximum secretion of ACTH in response to the polypeptides is significantly less than that for an extract of rat hypothalamic median eminence (HME) tissue. When added in combination with appropriate doses of HME extract, arg-vasopressin inhibits ACTH secretion. These findings suggest that the neurohypophysial polypeptides may be partial agonists of corticotropin secretion.

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