those for hypothalamic CRF[12]. In this context, it is interesting that Zimmerman has reported immunological evidence for another peptide in Brattleboro rats which cross-reacts weakly with anti-vasopressin[13] and may be related to the physiological CRF.

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GILLIES AND LOWRY REPLY—The above response to our paper[1] describes an experiment in which no difference was observed between the CRF activity of Brattleboro hypothalmic extracts and that of normal rats. As Brattleboro rats are deficient in vasopressin (VP), the authors conclude that VP is not CRF. However, our recent work with extracts of stalk-median eminence (SME) from Brattleboro rats[2] has added support to our idea that CRF is modulated VP. We found that Brattleboro SME contained approximately one fifth the CRF activity found in normal SME. Similar observations have been made using both isolated pituitary cells[3] and pituitary quarters[4].

Gel filtration of Brattleboro SME revealed only two small discrete peaks of CRF bioactivity corresponding in position and size to the Vo and Ns peaks found after chromatography of normal rat SME[1]. The third and major CRF peak found in normal rat SME, which coelutes with synthetic AVP and resembles AVP in our immunoassay and CRF bioassay, was absent from the Brattleboro chromatogram. Furthermore, addition of synthetic AVP in amounts equivalent to that found in normal SME (10 ng AVP per SME[5]) to extracts of Brattleboro SME completely restored the CRF activity to that of normals[2]. This observation has now been confirmed in a different assay system[4]. These results coupled with the facts that (1) we have consistently been able to combine the Vo and Ns CRF peaks from the chromatogram of normal SME with either the major AVP-CRF peak or equivalent amounts of synthetic AVP to restore full CRF bioactivity[1] and (2) no analogue of VP has been found with CRF bioactivity greater than AVP[5,6], suggest to us that we have ample support for our conclusion that the AVP-CRF peak is true vasopressin, and not merely related in structure to it.

The above response cites Zimmerman's preiiminary observation on the possibility of a vasopressin-like peptide in the Brattleboro rat which may be related to CRF. In a subsequent report[7], however, it was stated that homozygous Brattleboro rats lack VP and its associated neurophysin and the occasional staining of some cells in the SON and PVN of these rats with their antiserum to AVP "appears to be due to slight reactivity of this antiserum to oxytocin". It should also be noted that the antiserum used by Watkins[8] had 14% cross-reactivity with oxytocin and (8-arginine)-VP, so one cannot exclude the possibility of a positive immunohistochemical reaction in the SON and PVN of homozygous Brattleboro rats being due to oxytocin.

The immunocytochemical studies of Stillman *et al.*[9], showed that VP and its associated neurophysin are stimulated by adrenalectomy, and their content in pathways to the portal capillaries is regulated by glucocorticoids, suggesting an important role for VP in corticotropin release.

As Pearlmutter *et al.* state above, all agree that Brattleboro rats are able to respond to stress. However, results of *in vivo* experiments are contradictory as to whether this stress response, as measured by adrenal activity, is normal[10] or significantly subnormal[11–13] and, if an abnormality exists, whether it is at the hypothalamic, pituitary or adrenal level. We feel that many of the contradictions from *in vivo* experiments could be clarified, and a truer measure of CRF release in response to stress would be made, if ACTH release were the parameter measured, because supramaximal stress stimuli may lead to the release of CRF (the non-VP components in Brattleboro rats) and consequently ACTH, in amounts exceeding those necessary for maximal stimulation of the adrenal glands. This could explain why Yates *et al.*[12] observed that a deficiency in the stress response of homozygous Brattleboro rats was evident only when submaximal stresses were investigated.

We also suggest that the bioassay system used in *in vitro* experiments contributes to this controversy on the role of VP as a CRF. First, isolated cell systems in general, and especially the perfused isolated cell system, as bioassays for CRF are far more sensitive than those using pituitary halves or quarters [we were able to demonstrate a threefold potentiation of 0.025 Brattleboro SME per ml by 0.25 ng AVP per ml (ref. 2)]. This must account for the fact that we were able to observe discrete peaks of CRF bioactivity in the chromatogram of extracts of small numbers of rat SME, and identify one peak as VP[1]. Second, fresh excised rat anterior pituitaries contain significant amounts of VP[15] which disappear from the tissue only after incubation for 3 h, suggesting specific binding to receptors. Therefore, in bioassays which use freshly removed tissue or incubated tissue which has been previously stimulated with solutions containing VP, such as a hypothalamic extract (as in the bioassay system used by Pearlmutter *et al.*[6]), it is possible that no difference would be found between the CRF activity of Brattleboro and normal rat SME, as the VP component of CRF would already be in the tissue. It is interesting to note here that Buckingham and Leach[4], who use a CRF bioassay involving rat anterior pituitary quarters coupled with the cytochemical bioassay for ACTH[16], have reported diminished CRF bioactivity in Brattleboro SME. As well as the difference in the ACTH bioassays used, the discrepancy with the findings of Pearlmutter *et al.* may be due to the duration of static incubation of extracts with anterior pituitary tissue—15 min for Buckingham and Hodges[16] and 60 min for Pearlmutter *et al.*[6], compared with 2–3 min contact of stimuli with isolated cells in our perfused system. The net CRF bioactivity observed could depend on the breakdown of both CRF and ACTH in the incubation medium.

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