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Letters to the Editor

Ann Arbor, MI, May 23, 1985

Dear Editor,

We are writing with regard to the article by Dionne et al. [3], which is entitled 'Contrast Medium Cause the Apparent Increase in Beta-Endorphin Levels in Human CSF Following Brain Stimulation.'

The main conclusion of the article is derived from the observation that more beta-endorphin immunoreactivity is read following the injection of the contrast medium. The authors, therefore attribute the brain stimulation-induced elevation in endorphins which we have reported [1] to an artifact of the contrast medium on the assay. It is clear from their statement in the discussion that they assumed that our baseline sample, to which post-stimulation values were compared, preceded the injection of the contrast medium. In fact, however, our baseline samples were obtained *after the dye was injected* and *after the electrode was implanted*. In other words, what we called baseline is equivalent to their sample no. 3. Upon re-reading our original description, it is apparent that we may not have been sufficiently precise in our definition of baseline. We simply stated 'the first sample was obtained prior to the electrical stimulation and constituted the baseline control.' We did not specify that the sample contained the contrast medium. We do apologize for this omission; we must add, however, that our definition of baseline appeared to be the only experimentally sound one, as amply demonstrated by the Dionne et al. finding. So to clarify — in all our subjects the Conray dye was injected first and an X-ray obtained. The electrode was lowered to the target and another X-ray was obtained. The neurological status of the patient and his pain responsiveness was tested. One ml of CSF was withdrawn and discarded, to wash the dye off the walls of the cannula. Then the *baseline* sample was collected. This was immediately followed by electrical stimulation and collection of post-stimulation samples as described [1]. Thus, injection of the dye cannot explain our finding of elevated opioid levels upon stimulation.

Given that our baseline sample did contain the contrast medium, it should be noted here that under our assay conditions we read very low levels of beta-endorphin at baseline. Why should the dye not affect our radioimmunoassay? We believe it is a matter of dye concentration in the tube. We have used 100 μl of CSF/tube, whereas Dionne et al. used the equivalent of 850 μl. Thus we would be adding 8–9-fold less dye, and everything else being equal, we would get a great deal less interference. Further, the authors appear to have obtained the CSF very shortly post-dye, whereas in our case over an hour elapsed before we obtained the baseline.

It should also be noted that in the Science report [2], which the authors discuss, the CSF samples were first purified using 2 different techniques (biobead absorption

and ion exchange) prior to assay. One of the assays for opioid activity was a bioassay (vas deferens) and the CSF opioid effects were reversed by naloxone. This latter criterion would preclude non-specific dye effects.

In sum, our baseline measurement was post-dye, post-electrode implant and was separated from the other samples only by the actual brain stimulation. Thus, while we acknowledge the potential non-specific effects of the contrast medium, the elevations in opioids we have observed, using either radioimmunoassay or radioreceptor and bioassay, cannot be due to that artifact.

We feel that the Dionne et al. paper [3] does attract attention to an important potential problem especially when the CSF is concentrated prior to assay. The question remains, why did we observe post-stimulation elevations in endorphins and they did not (even on day 2 when no dye was present in the samples). The variability among reports is likely to be due to variation in the specific site of the implant. While we do not maintain that endorphin release is either necessary or sufficient to the production of stimulation-produced analgesia, we believe, based on our continued experience to date, that they are often correlated. Hopefully, future studies using newer and better purification techniques of the peptides will clarify the actual relationship between particular endogenous opioids and the production of analgesia.

Mental Health Research Institute,
University of Michigan,
Ann Arbor, MI 48109 (U.S.A.)

HUDA AKIL
DONALD E. RICHARDSON

References

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2 Akil, H., Richardson, D.E., Hughes, J. and Barchas, J.D., Enkephalin-like material elevated in ventricular cerebrospinal fluid of pain patients after analgesic focal stimulation, Science, 201 (1978) 468–475.

3 Dionne, R.A., Mueller, G.P., Young, R.F., Greenberg, R.P., Hargreaves, K.M., Gracely, R. and Dubner, R., Contrast medium causes the apparent increase in beta-endorphin levels in human cerebrospinal fluid following brain stimulation, Pain, 20 (1984) 313–321.