Circulating opioid peptides during water immersion in normal man

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(Received 3 July 1987; accepted 21 July 1987)

SUMMARY
1. This study was designed to evaluate variations in plasma β-endorphin, methionine-enkephalin, adrenocorticotropic hormone and serum prolactin in healthy volunteers during head-out water immersion.
2. Water immersion induced an increase in methionine-enkephalin plasma levels, which was associated with a significant fall in mean arterial pressure and heart rate.
3. Conversely, a suppression of plasma β-endorphin, adrenocorticotropic hormone and serum prolactin was detected during water immersion.
4. We suggest that a dopaminergic inhibitory control mechanism may be involved in regulating circulating levels of β-endorphin, adrenocorticotropic hormone and prolactin in normal subjects undergoing extracellular fluid volume expansion produced by water immersion.

Key words: adrenocorticotropic hormone, β-endorphin, methionine-enkephalin, prolactin, water immersion.

INTRODUCTION
Systemic administration of morphine-like compounds [1, 2] and stimulation of the atrial volume receptors by distension [3, 4] may induce quite similar haemodynamic responses. In fact, a fall in blood pressure, bradycardia and decreased systemic vascular resistance have been frequently described in both conditions. It seems possible that a common neurotransmitter could be mediating these cardiovascular events.

Water immersion to the neck (WI) may represent a useful method for stimulating low pressure receptors [4, 5] discharging in areas of the brain stem which are intimately involved in blood pressure regulation [6]; both opioid-containing nerve terminals and opiate receptors have been identified in these areas [7, 8].

Circulating endogenous opioid behaviour has been evaluated in this study in order to clarify a possible role of these peptides in determining the haemodynamic and hormonal events elicited by atrial distension during WI.

MATERIALS AND METHODS
The subjects in this study were eight male healthy volunteers, with a mean age of 23 years, who had given informed consent. They did not use tobacco, were not taking any medications, and were asked to refrain from the use of alcohol and other drugs for 24 h before the study.

The immersion study started at 08.00 hours after an overnight fast. As previously described [9], the subjects sat quietly outside of the immersion tank for 1 h (pre-immersion study). Then they stepped into the immersion tank and sat on a stool with water to the neck at constant temperature (34.5 ± 0.5°C) with their arms outside of the tank. The subjects remained in this tank for 2 h. Arterial blood pressure was measured non-invasively every 10 min with an automatic blood pressure monitor (Arteriosonde Roche model 1225); heart rate was recorded continuously on a Battaglia-Rangoni polygraph. All blood samples were taken through an indwelling Teflon catheter (with an accompanying Teflon stylet) in an antecubital vein. Venous blood was drawn into cold Vacutainer tube containing a mixture of ethylenediaminetetra-acetate and enzymatic inhibitor aprotinin (Tzalol, Bayopharm), at 08.30, 09.00 (pre-immersion period), 10.00 and 11.00 hours (immersion period). Venous blood was immediately centrifuged at 0–4°C. At all time points, 3.5 ml of plasma...
was obtained and divided as follows: two 1.5 ml portions for determination of immunoreactive total \( \beta \)-endorphin and immunoreactive methionine-enkephalin in duplicate, one 500 \( \mu l \) portion for measurement of adrenocorticotropic hormone (ACTH) in triplicate and 500 \( \mu l \) of serum for assay of prolactin (PRL) in duplicate. All plasma samples were frozen within 10 min of collection and were stored at \(-70^\circ\)C.

Water immersion was performed at the same time of the day to avoid influences related to circadian rhythm of the hormones being studied. At a separate time, the same normal subjects came to the laboratory and rested for 3 h outside of the immersion tank; venous blood was drawn, as described above, at 08.30, 09.00, 10.00 and 11.00 hours for measurement of \( \beta \)-endorphin, methionine-enkephalin, ACTH and PRL.

\( \beta \)-Endorphin, methionine-enkephalin, ACTH and PRL were measured by radioimmunoassay. \( \beta \)-Endorphin was evaluated with the Allegro\textsuperscript{TM} \( \beta \)-endorphin immunoassay system (Nichols Institute, San Juan Capistrano, CA, U.S.A.); this assay has a calculated sensitivity of 10 pg/ml and has a cross-reactivity of 16% with \( \beta \)-lipotrophin. The coefficients of variation for inter- and intra-assay determinations were 10% and 7% respectively. Methionine-enkephalin was determined using the radioimmunoassay kit from Immuno Nuclear Corporation (MN, U.S.A.), according to the original method of Clement-Jones et al. [10]; this assay has a calculated sensitivity of 30 pg/ml and has a cross-reactivity of 2.8% with leucine-enkephalin. The coefficients of variation for inter- and intra-assay were 7.2% and 3.8% respectively. The intra-assay coefficients of variation for \( \beta \)-endorphin and methionine-enkephalin were calculated from repeated determinations on each of two quality control sera in a single assay (n = 20 and n = 15 respectively). The interassay coefficients of variation were calculated from data obtained for two quality control sera assayed during a 1 month period (n = 35 and n = 30, respectively). Plasma immunoreactive ACTH was measured with a IRE-Medgenix ACTH100-RIA-kit (Brussels, Belgium) with a sensitivity of 12 pg/ml and inter- and intra-assay coefficients of variation of 7% and 5% respectively. Serum PRL was determined with a Serono RIA-kit (Milan, Italy).

Statistical analysis included paired Student's \( t \)-test and analysis of variance. Mean values, \( \pm \)SEM as an index of dispersion, are presented.

### RESULTS

Mean arterial pressure (diastolic plus one-third of pulse pressure) during the pre-immersion period was 97 \( \pm \)3 mmHg and showed a significant, albeit slight, decrease (92 \( \pm \)2 mmHg; \( P < 0.025 \)) during WI; heart rate fell during WI from 75 \( \pm \)2 to 69 \( \pm \)2 beats/min (\( P < 0.005 \)). A significant increase in plasma levels of methionine-enkephalin occurred during WI (from 101 \( \pm \)7.3 to a peak value of 145 \( \pm \)5.6 pg/ml; \( P < 0.001 \)).

In contrast, a significant suppression of circulating \( \beta \)-endorphin was found during WI (from 51 \( \pm \)2 to a nadir of 30 \( \pm \)1 pg/ml; \( P < 0.001 \)). Furthermore, a profound reduction in plasma ACTH (from 96 \( \pm \)19.6 to 56 \( \pm \)15 pg/ml; \( P < 0.01 \)) and serum PRL (from 5 \( \pm \)0.5 to 3.5 \( \pm \)0.2 ng/ml; \( P < 0.005 \)) was detected during WI.

No significant changes in circulating \( \beta \)-endorphin, methionine-enkephalin and PRL were found in our subjects during the period of quiet sitting outside of the immersion tank. In comparison with these control values, the changes in circulating levels of methionine-enkephalin, \( \beta \)-endorphin and PRL during WI were statistically significant (\( P < 0.01 \)).

A normal circadian fall in ACTH plasma levels was detected in our subjects during the control period; on the other hand, the suppression of ACTH, seen during WI was significantly different (\( P < 0.01 \)) from that observed during the control period. Fig. 1 shows the time course of the hormones studied during both the control period and WI.

### DISCUSSION

In this study we have shown that stimulation of the cardio-pulmonary mechanoreceptors by central hypervolaemia...
induces a release of the opioid peptide methionine-enkephalin with a concomitant reduction in blood pressure and heart rate in man undergoing WI. Several studies suggest that peptides related to enkephalins generally cause, when given intravenously, peripheral vasodilatation, a fall in blood pressure and bradycardia [11, 12]. Whether blood pressure and heart rate reduction during WI are direct effects of elevated levels of methionine-enkephalin or the result of opioid-induced changes in baroreflex sensitivity [13] cannot be elucidated from this study.

There is a further haemodynamic event induced by WI that is not fully explained and which should be carefully considered: the failure of the human to respond to immersion with a tachycardia (Bainbridge reflex) despite a substantial increase in both cardiac filling and atrial pressure [5, 14, 15]. In fact, terminals of noradrenergic neurons have specific opioid receptors and activation of these presynaptic receptors by opioids could lead to a reduction in the amount of transmitter released [16]. A significant reduction in the plasma level of noradrenaline has been observed during central hypervolaemia produced by WI [17, 18], thus further suggesting a possible opioid control of noradrenaline release; a methionine-enkephalin-induced reduction of sympathetic activity [19] might explain this unexpected bradycardic event.

The WI-induced suppression of β-endorphin is a surprising but not unexpected phenomenon. In general, dopamine inhibits prolactin and ACTH release [20] and this concept has been confirmed in normal man undergoing WI [21, 22]. In addition, agonists of dopamine receptors attenuate the stress-induced release of β-endorphin and ACTH and completely prevent the stimulated release of PRL [23, 24]. There is also evidence that dopamine regulates the release of β-endorphin [23] which is known to be secreted concomitantly with ACTH [20].

We and others have demonstrated that WI induces a significant increase in dopamine plasma levels [17, 18]; in view of the fact that dopamine has been shown to exert a tonic inhibitory influence on β-endorphin release, we suggest that increased dopaminergic activity might explain the significant suppression of plasma β-endorphin, ACTH and PRL seen during central hypervolaemia produced by WI in our normal subjects. This hypothesis explains the demonstration that dopaminergic antagonist administration may reverse the significant β-endorphin suppression observed during WI (unpublished work). In the present study no changes in circulating opioid peptides, ACTH or PRL were found in our subjects during the period of quiet sitting outside of the immersion tank; this suggests that the opioid system is largely quiescent under basal conditions [25] and becomes active during physiological manoeuvres such as extracellular fluid volume expansion.

In conclusion, this study shows that central hypervolaemia produced by WI induces a significant increase in circulating methionine-enkephalin levels, thus contributing to the explanation of several haemodynamic events currently observed during WI. On the other hand, a significant suppression of circulating β-endorphin levels is seen during WI; an increased dopaminergic activity may account for β-endorphin, ACTH and PRL suppression.

REFERENCES
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