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Elcatonin Raises Levels of Vasoactive Intestinal Peptide in Human Plasma

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Abstract

Elcatonin, used for treatment of hypercalcaemia, Paget's disease and osteoporosis, causes flushing of the face and hands. To determine whether this was because of increased levels of vasoactive intestinal peptide, which is known to induce vasodilation, the effect of elcatonin on the plasma levels of vasoactive intestinal peptide was studied in five healthy volunteers.

After a single intramuscular administration of elcatonin (20 int. units), peak plasma elcatonin levels (approx. 30 pg mL^{-1}) were achieved 30 min after injection. Plasma vasoactive intestinal peptide concentrations rose similarly with peak levels of about 17 pg mL^{-1} after 30 min. Side-effects such as cutaneous flushing (most obvious in the face and hands) occurred to an extent dependent on the amount of elcatonin administered, and declined over 45 min in parallel with the fate of plasma vasoactive intestinal peptide.

The side-effects of elcatonin, especially cutaneous flushing, seem to be closely connected with vasoactive intestinal peptide.

The hypocalcaemic peptide calcitonin was discovered by Copp & Devidson (1961) and has been isolated from the thyroid glands of various species. The amino acid sequences of porcine, bovine, ovine, human (Neher et al 1968), salmon, eel (Otani et al 1976) and rat calcitonin have been determined. Maier et al (1974) reported that the amino group at the *N* terminus of human calcitonin is not necessary for the hypocalcaemic action. Morikawa et al (1976) synthesized $Asu^{1,7}$-eel calcitonin in which the S–S bond between amino acids 1 and 7 of the calcitonin molecule was replaced by a C–C bond. $Asu^{1,7}$-eel calcitonin (elcatonin) is hypocalcaemic, and has been reported to be more stable than eel calcitonin under physiological conditions (Yamauchi et al 1977). Elcatonin, which is frequently used in Japan for the treatment of hyper-calcaemia, Paget's disease and osteoporosis, frequently causes nausea and flushing of the face and hands.

Vasoactive intestinal peptide (VIP) is a 28 amino acid neuropeptide initially isolated from intestinal tissue by Said & Mutt (1970). It is widely distributed in the central and peripheral nervous system (Fahrenkrug 1993), and induces vasodilation in many vascular beds including the peripheral systemic and splanchnic beds and the cerebral arteries. Intravenous infusion of VIP caused cutaneous flushing particularly in the head region but did not cause nausea (Domschke et al 1978).

In this study, we examined the plasma concentrations of VIP-like immunoreactive substance (VIP-IS) before and after elcatonin administration in healthy subjects.

Materials and Methods

Subjects

Five healthy male volunteers aged 23 to 48 years (median 32 years) weighing 56–66 kg (median 60 kg) participated in the study. Each subject received information about the pure scientific purpose of the study, which was approved by the Ethics Committee at Oita Medical University, and gave informed consent. None received any medication other than elcatonin during the study.

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Study schedule

Elcatonin (Elcitonin Injection, Asahi Chemical Industry Co, Ltd, Tokyo, Japan) in 154 mM NaCl (saline) at a dose of 20 int. units, or control saline was administered intramuscularly. Venous blood samples (10 mL) were taken from a forearm vein before and 10, 20, 30, 45, 60, 90 and 120 min after the administration of elcatonin. The study was performed from 1400 to 1600 h.

Preparation of plasma extracts

The blood samples were placed in chilled tubes (4°C) containing aprotinin (500 kallikrein inhibitor units mL^{-1}) and EDTA (1·2 mg mL^{-1}). After centrifugation (1670 *g*, 4°C, 20 min), plasma samples were diluted fivefold with 4% acetic acid, pH 4·0, and loaded onto C_{18} reversed-phase cartridges (Sep-Pak C_{18}; Millipore Corp., Milford, MA, USA). After washing with 4% acetic acid, VIP-IS in plasma was eluted with 70% acetonitrile in 0·5% acetic acid, pH 4·0. Eluates were concentrated by spin-vacuum evaporation, lyophilized and stored until used. When 10 pg mL^{-1} of synthetic VIP was added to hormone-free plasma prepared by the method of Tai & Chey (1978), the recovery of VIP using this extraction procedure was $94 \pm 6\%$ ($n = 6$).

Enzyme immunoassays (EIAs) for VIP and elcatonin

EIA for VIP was performed as previously described (Takeyama et al 1990). Antiserum (604/001) purchased from UCB Bioproducts SA (Alleud, Belgium) was specific to the central region of VIP as examined with synthetic VIP and its fragments. The VIP fragment (position 10–28) was labelled with β-D-galactosidase. EIA was performed by the delayed addition method. Separation of bound and free materials was performed by the

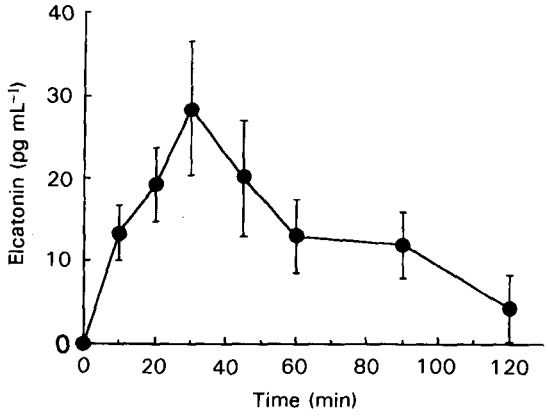

FIG. 1. Plasma elcatonin levels after a single intramuscular administration of elcatonin. Each point represents the mean ± s.d. of concentrations in five volunteers

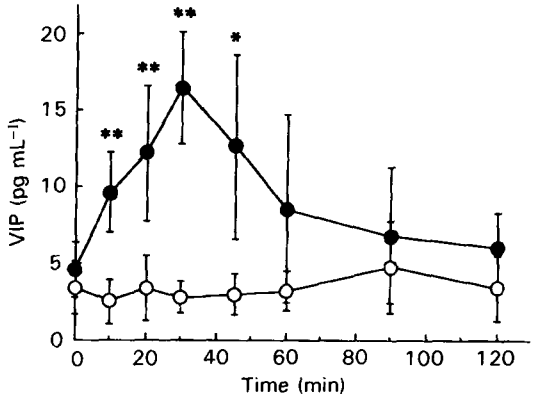

FIG. 2. Plasma vasoactive intestinal peptide (VIP) levels after intramuscular administration of elcatonin (●) and saline (○, control). Each point represents the mean ± s.d. of concentrations in five volunteers. $^{**}P < 0.01$, $^{*}P < 0.05$ compared with control.

double antibody solid phase method. The detection unit of VIP was 0.1 fmol per well. EIA for elcatonin was essentially the same, except that the antiserum for elcatonin (i674, UCB Bioproducts SA, Alleud, Belgium) was used (Takeyama et al 1995).

HPLC of plasma extracts

HPLC was performed using a reversed-phase C_{18} column (Cosmosil 5C18; Nacalai Tesque, Kyoto, Japan). The column was equilibrated with 0·1% trifluoroacetic acid, plasma extracts were applied to the column, and VIP-related material was eluted with a linear gradient of acetonitrile (10% in 6 min and 10–45% in 35 min) in 0·1% trifluoroacetic acid. Synthetic VIP and its sulphoxide (VIP(O)) were applied to the column under the same conditions. The flow rate was 1 mL min^{-1}, and the fraction size was 1 mL. Each fraction was concentrated by spin-vacuum evaporation, lyophilized, and the residues were submitted to EIA.

Statistical analysis

All values are expressed as means ± s.d. Comparisons of mean values were made by one-way analysis of variance. A value of $P < 0.05$ was regarded as significant.

Results

The profiles of average plasma elcatonin concentration against time after intramuscular administration of elcatonin are shown in Fig. 1. The maximum plasma level, approximately 30 pg mL^{-1}, was reached 30 min after administration.

Intramuscular injection of saline did not alter plasma VIP concentrations (Fig. 2), whereas elcatonin caused an approximately fivefold increase with a peak at 30 min after injection (Fig. 2). Elcatonin caused nausea and cutaneous flushing, the latter particularly in the face and hands; the flushing declined in parallel with the decrease in plasma VIP, whereas nausea continued after this decrease. Blood pressure and pulse rate did not change after elcatonin administration.

The main VIP-IS in plasma extracts were eluted with the same elution volume as was the synthetic VIP, with approximately 30% acetonitrile, and there was a minor peak at the same elution volume of VIP sulphoxide (VIP(O)) with about 25%

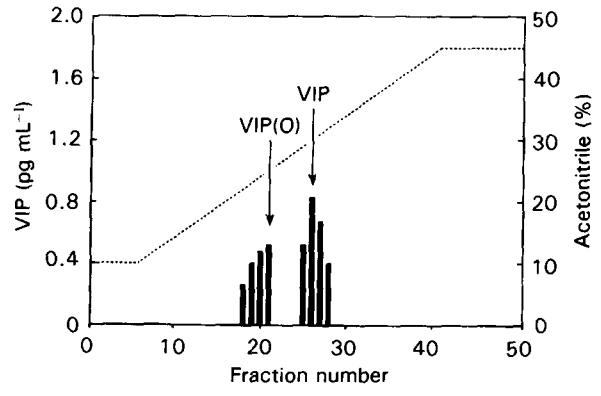

FIG. 3. HPLC elution profile of plasma extract. The arrows indicate the elution regions of synthetic vasoactive intestinal peptide (VIP) and VIP sulphoxide (VIP(O)). Percentage acetonitrile is indicated by the dotted line.

acetonitrile (Fig. 3). The assay of the plasma extracts was therefore considered to be specific for VIP.

Discussion

Side-effects of calcitonin such as flushing and vomiting are well described by Luengo et al (1990), who reported that 85% of patients, who completed the 1-year follow-up period, presented with cutaneous flushing such as redness and hot flushes of the face, whereas only 47% of patients presented with various degree of nausea. Because elcatonin is frequently used in Japan to treat hypercalcaemia, osteoporosis and Paget's disease, we studied the effect of elcatonin on VIP concentrations in plasma.

The parallel rise of VIP with elcatonin concentration suggests a causal relationship. Thus peripheral release of VIP seems to explain the cutaneous flushing, which disappeared in parallel with the VIP levels. The other side-effect, nausea, on the other hand, continued after the decrease in plasma VIP levels.

We do not know the precise mechanism whereby VIP levels are increased, but speculate on two possibilities. One is that elcatonin stimulates 5-hydroxytryptamine (5-HT) neurons (Nakhla & Mujamdar 1978) and elevated 5-HT could produce a marked release of VIP (Eklund et al 1980). At the same time,

elevated 5-HT would be partly responsible for nausea. Another possibility is that elcatonin directly stimulates peripheral VIP neurons, producing a marked release of VIP, causing cutaneous flushing. This study suggests the close connection of VIP with the side-effects of elcatonin, especially cutaneous flushing, although the precise mechanism how VIP levels are increased remains an open question.

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