SHORT COMMUNICATION

Immunoreactive substance P in human plasma: response to changes in posture and sodium balance

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Summary
1. In healthy volunteers plasma concentrations of immunoreactive substance P were measured in response to changes in posture and dietary salt intake.
2. In 44 subjects plasma immunoreactive substance P was 168 ± 31 pmol/l when subjects were supine and 401 ± 51 pmol/l (P < 0.001) when they were ambulant.
3. Measurement of supine plasma immunoreactive substance P at 6 h intervals gave a mean value of 240 ± 39 pmol/l at 14.00 hours and a lowest value of 76 ± 9 pmol/l at 02.00 hours.
4. In eight healthy subjects plasma immunoreactive substance P rose only slightly from 169 ± 41 pmol/l, on a sodium intake ad lib., to 244 ± 45 pmol/l by day 4 of dietary sodium restriction (35 mmol/day) and significantly fell to 51 ± 20 pmol/l (P < 0.001) by day 4 of high sodium intake (350 mmol/day).
5. Although exogenous substance P was shown to be natriuretic in dog and rat, the present results do not favour a role of endogenous substance P as a circulating natriuretic factor in man.

Key words: diurnal variation, immunoreactive substance P, postural changes, sodium balance.

Introduction
Substance P, an undecapeptide (Chang & Leeman, 1970; Studer, Trzeciak & Lergier, 1973) originally isolated from brain and intestine (von Euler & Gaddum, 1931), is present in hypothalamic tissue of various species (Powell, Leeman, Tregear, Niall & Potts, 1973) and is found in high concentrations in the pituitary gland (Yanaihara, Sato, Hirohashi, Sakagami, Yamamoto, Hashimoto, Yanaihara, Aba & Kaneko, 1976). Experimental data suggest that this neurotransmitter may play a role in the regulation of water balance (de Caro, Massi & Micossi, 1978; Strumillo-Dyba, 1978; Gullner, Campbell & Pettinger, 1979) and may also participate in the control of renin release (Güllner & Barter, 1979). At low concentrations, substance P increases blood flow and lowers arterial blood pressure (Fisher, Humphries & Folkers, 1974) and is a potent natriuretic peptide when infused into the renal artery (Macfarlane, Mills & Ward, 1974) or systemically into the jugular vein (Flachskampf, Düssing & Kramer, 1980). This natriuresis appears to result predominantly from decreased proximal-tubular sodium absorption (Arendshorst, Cook & Mills, 1976; Flachskampf et al., 1980). However, at present there is little information on the plasma concentrations of substance P in man (Nilsson, Pernow, Fisher & Folkers, 1975; Yanaihara et al., 1976) and no data concerning its response to physiological stimuli have been reported so far. This study was therefore undertaken to investigate immunoreactive substance P in human plasma under physiological conditions, i.e. its diurnal variation and response to changes in posture and dietary salt intake.

Materials and methods
Plasma concentrations of immunoreactive substance P were determined in 14 healthy...
volunteers (seven female and seven male subjects, aged 19–35 years) at 08.00 hours after an overnight fast, in the supine position, on the next morning at 08.00 hours after 2 h of upright posture and again at 11.00 hours when they were ambulant. In addition, in five healthy male subjects plasma concentrations of immunoreactive substance P were determined at 6 h intervals, with subjects supine for 24 h.

In eight subjects plasma concentrations of immunoreactive substance P were measured after an overnight fast at 08.00 hours during sodium intake ad lib., on day 4 of sodium restriction (35 mmol/day) and again on day 4 of high sodium intake (350 mmol/day).

Blood was collected without tourniquet from the antecubital vein into plastic tubes containing phenanthroline and EDTA. After centrifugation, plasma was deproteinized with ethanol, acidified with hydrochloric acid (10 mmol/l) and extracted with methylene chloride. The aqueous phase was then freeze-dried and dissolved in bovine serum albumin/peptone buffer before assay. Mean recovery of added 125I-labelled substance P was 43 ± 2%. Radioimmunoassay of substance P was performed with a rabbit anti-substance P antiserum and 125I-labelled substance P (Immuno Nuclear Corporation, Stillwater, Minnesota, U.S.A.). This antibody cross-reacts with other neurotransmitters as follows: methionine-enkephalin, leucine-enkephalin, eledoisin and physalaemin <0.002%; /-?-endorphin 0.008%. Unlabelled substance P was purchased from Serva GmbH, Heidelberg, F.R.G.

Mean binding of 125I-labelled substance P to the antibody was 60.3 ± 1.7% and the lower detection limit of the assay was 14 pmol/l. Of 300 pmol of substance P added to plasma with known amounts of immunoreactive substance P, 86–108% was recovered. Serial dilutions of plasma were assayed and were found to parallel the standard curve. When identical samples were assayed repeatedly the intra-assay variability was ±8.5% and the coefficient of variation of the interassay variability was 9.8%. All determinations were performed in duplicate. Results were corrected for recovery and expressed as pmol/l. Statistical analysis was performed with a paired Student’s t-test and results are expressed as means ± SEM.

Results

In 14 subjects supine plasma concentrations of immunoreactive substance P at 08.00 hours averaged 168 ± 31 pmol/l (females: 171 ± 39; males: 164 ± 53 pmol/l), a value similar to that reported by Nilsson et al. (1975) and Yanaihara et al. (1976). Mean plasma concentration of immunoreactive substance P in these subjects after 2 h of ambulation at 08.00 hours was 401 ± 51 pmol/l (Fig. 1a) and after 3 h of ambulation at 11.00 hours was 378 ± 29 pmol/l (P < 0.001). In five additional subjects a diurnal variation of supine plasma immunoreactive substance P was observed: mean plasma concentration at 08.00 hours was 129 ± 18 pmol/l and rose to a value of 240 ± 39 pmol/l at 14.00 hours (P < 0.05). It declined to 156 ± 18 pmol/l at 20.00 hours and to 76 ± 9 pmol/l at 2.00 hours (P < 0.01 as compared with values at 08.00 hours. At 08.00 hours mean plasma concentration again returned to 162 ± 25 pmol/l.

On day 4 of low salt intake, no significant changes in the concentration of immunoreactive substance P in plasma of eight subjects were observed (244 ± 45 vs 169 ± 41 pmol/l on sodium intake ad lib.), and it decreased significantly after 4 days on a high salt intake (51 ± 20 pmol/l; P < 0.001) (Fig. 1b).

Discussion

Although substance P is a potent natriuretic peptide when infused systemically (Flachskampf et al., 1980) or into the renal artery of the rat (Arendshorst et al., 1976), the present results do not favour a role of endogenous substance P as a circulating natriuretic factor in man under the present experimental conditions.

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Effects of posture and salt balance on plasma substance P

References


