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Effects of intradermal injection of atrial natriuretic peptide

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Atrial natriuretic peptide (ANP) causes mast cell degranulation in rats *in vivo* and *in vitro* but is bronchodilator in humans. The aim of this study was to investigate the wheal and flare dose-response to intradermal injection of α-human ANP in normal humans. Eight normal subjects received five 30 μl injections containing 1, 10, 39, 78, 117 pmol ANP and one each of normal saline, histamine 675 pmol and substance P 30 pmol. Maximum ANP flare response was greater but not significantly than that to saline at 1.55 ± 0.6 (mean ± s.e. mean) compared with 0.42 ± 0.17 cm^2, but much less than to histamine 9.86 ± 0.97 or to substance P 12.5 ± 1.2. Maximum ANP wheal response was significantly greater than that to saline at 0.38 ± 0.08 compared with 0.18 ± 0.05 cm^2 (difference between means 0.20, 95% CI 0.05, 0.35), but much less than to histamine 0.75 ± 0.06 or to substance P 1.05 ± 0.08 cm^2. No dose-response to ANP was demonstrated, though responses to the highest dose differed significantly from those to the lowest dose studied. We conclude that human cutaneous responses to ANP differ from those of animals and that the skin is less responsive than other tissues in humans.

Keywords atrial natriuretic peptide human skin flare wheal

Introduction

Human ANP is a 28 amino acid peptide hormone with vasodilator, natriuretic and diuretic properties which has recently been shown to have bronchodilator activity when given by infusion [1] or inhalation [2]. However, *in vitro* ANP has recently been shown to release histamine from rat peritoneal mast cells and to increase rat skin vascular permeability *in vivo* over a 3–4 log dose range [3]. Other peptides such as substance P and other tachykinins release histamine from mast cells *in vitro* and lead to cutaneous wheal and flare responses in man [4]. If ANP caused mast cell degranulation in humans then it would be expected to have adverse consequences when administered to asthmatic patients. The aim of this study was to examine whether injection of human ANP causes cutaneous mast cell responses in normal volunteers.

In this preliminary study we tested the hypothesis that ANP induces wheal and flare responses in a dose-dependent manner in human skin similar to its effect in animals. We compared responses with those elicited by previously selected doses of substances P and histamine [5].

Methods

Eight normal subjects, mean age 37 (range 30–45) years, were studied on one occasion. The study was approved by the Ethics Committee of the Royal Postgraduate Medical School and Hammersmith Hospital, and all volunteers gave written informed consent. Intradermal injections were made in a volume of 30 μl with a 29 gauge needle. Stock solutions of α-human ANP (Bachem Inc, Torrance-California), 3.25×10^{-5} M in distilled water and substance P 0.1 mM (Sigma, Poole, UK) in 17 mM acetic acid in isotonic saline were prepared [5]. Final dilutions of ANP, substance P and histamine acid phosphate were made in sterile isotonic saline immediately before use.

Five intradermal injections of 30 μl ANP 3.25×10^{-8}, 3.25×10^{-7}, 1.3×10^{-6}, 2.6×10^{-6}, 3.9×10^{-6} M (containing 1, 10, 39, 78, 117 pmol) and one each of saline, histamine 2.25×10^{-5} M (675 pmol) and substance P 1×10^{-6} M (30 pmol) were given coded and in random order. Four injections were given to each forearm. Both subject and observer recording the results were unaware of the code. Flare area and wheal area were outlined with a ball-point pen at 5 and 15 min respectively. A permanent record was

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made by transferring the outline onto 1 mm graph paper using transparent adhesive tape (Sellotape).

Statistical methods

Results were expressed as mean ± s.e. mean (unless otherwise indicated). Non-parametric Wilcoxon signed rank tests were used to compare changes in flare and wheal surface area between treatments. A value of $P < 0.05$ was considered statistically significant.

Results

The intradermal injections of saline and ANP caused transient discomfort lasting less than 10 s in all subjects. Histamine and substance P caused pain and itch in six subjects. Subjects did not distingush injections containing ANP from others. No systemic side effects occurred. Flare responses (mean ± s.e. mean) to ANP 1, 10, 39, 78 and 117 pmol were 0.33 ± 0.11, 0.70 ± 0.26, 0.52 ± 0.14, 0.68 ± 0.20 and 1.55 ± 0.57 cm^2 and wheal responses were 0.22 ± 0.05, 0.21 ± 0.06, 0.22 ± 0.06, 0.27 ± 0.08 and 0.38 ± 0.08 cm^2, respectively. There were no significant differences between ANP 1, 10, 39, and 78 pmol and saline control, but maximum ANP wheal response was significantly greater than that to saline (difference between means 0.20, 95% CI 0.05, 0.35, $P < 0.05$). Flare and wheal responses to maximum ANP were significantly greater than those to ANP 1 pmol; difference between means 1.22, 95% CI 0.13, 2.31 for flare ($P < 0.02$) and 0.16, 95% CI 0.03, 0.24 cm^2 for wheal ($P < 0.05$). Flare and wheal responses were 9.86 ± 0.97 and 0.75 ± 0.06 cm^2 for histamine and 12.5 ± 1.2 and 1.05 ± 0.08 cm^2 for substance P. These responses were significantly greater than those to ANP ($P < 0.02$, Figure 1).

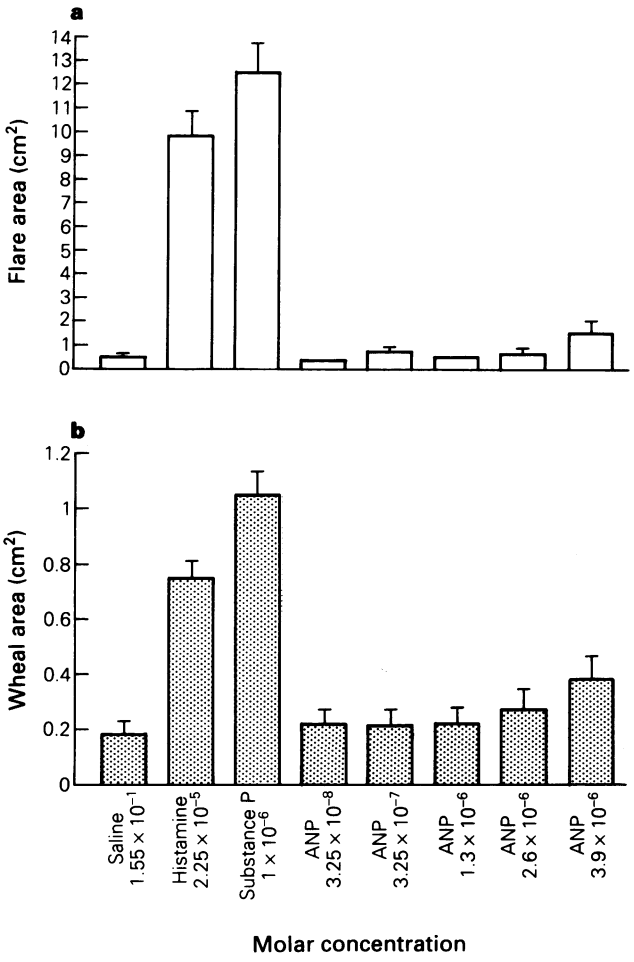

Figure 1 a) Flare and b) wheal response areas in cm^2 (mean ± s.e. mean) to intradermal injection of 30 µl normal saline (0.9%), histamine (675 pmol), substance P (30 pmol) and ANP (1, 10, 39, 78, 117 pmol).

Discussion

This is the first study to report the effects of intradermal α-human ANP in man. Small flare and wheal responses were produced. Only the wheal response to the highest dose of ANP differed significantly from that to saline. Similarly, only flare and wheal responses to the highest dose of ANP differed from those to the lowest dose (2 log units less). Thus, we failed to define a dose-response in this preliminary study. Cost has so far prohibited the use of higher doses of ANP.

The highest concentrations of ANP used were fourfold greater than those producing increased permeability in rat skin (1×10^{-6} M) [3]. Furthermore dose-dependent histamine release from rat isolated peritoneal mast cells *in vitro* occurs with atrial peptides [ANP(1–28), ANP(3–28) and ANP(5–28)] at concentrations starting at 1×10^{-7} M [3]. Finally, high dose infusion of ANP in man produced respiratory, metabolic and marked cardiovascular responses at serum concentrations of $5.7 \times 10^{-8} - 9.6 \times 10^{-7}$ M [1]. Infusion of similar doses produced significant changes in cutaneous blood flow [6, 7]. It is unclear why human skin appears less responsive to ANP than other tissues but this may relate to enhanced activity of cutaneous endopeptidases.

We obtained significantly greater cutaneous responses to histamine and substance P, although the molar concentrations of ANP used were comparable. Responses to histamine and substance P were similar to those obtained by Foreman *et al.* [4] and ourselves in a previous study [5]. We measured wheal area as opposed to volume, but previous work has shown good correlation between the two [8] and it is unlikely that our findings would have been altered by determination of skin thickness.

It is possible that different forms of ANP might have different activities but α-human ANP appears the most relevant for study in man and was the most active *in vitro* [3]. It is unclear to what extent human cutaneous mast cell responses depend on specific receptors and to what extent they are determined by

the peptides basic nature [4]. We have shown that the response in humans is reduced compared with that in rats. Furthermore, cutaneous wheal and flare responses are minimal at ANP concentrations which elicit marked cardiovascular effects in humans [1]. The dose-dependent bronchodilator effects of ANP in animals [9, 10] and humans [1, 2, 11, 12], without any suggestion of pulmonary mast cell degranulation, are consistent with our finding of reduced mast cell response to ANP in human skin *in vivo*.

References

1 Angus RM, McCallum MJ, Hulks G, Thomson NC. Bronchodilator, cardiovascular, and cyclic guanylyl monophosphate response to high-dose infused atrial natriuretic peptide in asthma. *Am Rev Respir Dis* 1993; **147**: 1122–1125.

2 Hulks G, Thomson NC. Inhaled atrial natriuretic peptide and asthmatic airways. *Br Med J* 1992; **304**: 1156.

3 Opgenorth TJ, Budzik GP, Mollison KW, *et al.* Atrial peptides induce mast cell histamine release. *Peptides* 1990; **11**: 1003–1007.

4 Foreman J, Jordan C. Histamine release and vascular changes induced by neuropeptides. *Agents Actions* 1983; **13**: 105–116.

5 Barnes PJ, Brown MJ, Dollery CT, Fuller RW, Heavey DJ, Ind PW. Histamine is released from skin by substance P but does not act as the final vasodilator in the axon reflex. *Br J Pharmacol* 1986; **88**: 741–745.

6 Bussien JP, Biollaz J, Waeber B, *et al.* Dose-dependent effect of atrial natriuretic peptide on blood pressure, heart rate, and skin blood flow of normal volunteers. *J Cardiovasc Pharmacol* 1986; **8**: 216–220.

7 Webb DJ, Benjamin N, Allen MJ, Brown J, O'Flynn M, Cockcroft JR. Vascular responses to local atrial natriuretic peptide infusion in man. *Br J Clin Pharmacol* 1988; **26**: 245–251.

8 Cook J, Shuster S. Histamine weal formation and absorption in man. *Br J Pharmacol* 1980; **69**: 579–585.

9 Ishii K, Murad F. ANP relaxes bovine tracheal smooth muscle and increases cGMP. *Am J Physiol* 1989; **256**: 495–500.

10 Robichaud A, Michoud MC, Saunier C, *et al.* Effect of atrial natriuretic peptide on bronchial tone in anaesthetised rabbits. *Peptides* 1993; **14**: 1325–1330.

11 McAlpine LG, Hulks G, Thomson NC. Effect of atrial natriuretic peptide given by intravenous infusion on bronchoconstriction induced by ultrasonically nebulized distilled water (fog). *Am Rev Respir Dis* 1992; **146**: 912–915.

12 Westendorp RG, Roos AN, van der Hoeven HG, *et al.* Atrial natriuretic peptide improves pulmonary gas exchange in subjects exposed to hypoxia. *Am Rev Respir Dis* 1993; **148**: 304–309.

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