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EFFECTS OF TEMPERATURE AND OSMOLALITY ON THE RELEASE OF ATRIAL NATRIURETIC PEPTIDE

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In isolated rat atria a 10°C increase in temperature approximately doubled the output of atrial natriuretic peptide during relaxation and stretch. The effect was not due to the increased rate of contraction. Increasing the osmolality of the superfusate within the physiological range (290 to 320 m osmols) with sodium, potassium or glucose had no appreciable effect on the release of atrial natriuretic peptide.

Stretching of the atrial wall appears to be the main physiological stimulus for the exocytosis of atrial natriuretic peptide and can be demonstrated in vitro (1). In addition to stretch, two other physical factors have been reported to affect the release of atrial natriuretic peptide: temperature and osmolality. Bilder et al (2) found that a reduction in bath temperature decreased the rate of secretion of atrial natriuretic peptide from isolated preparations of rat atrium, although it was not clear to those authors to what extent this was due to a reduction in the frequency of contraction. An increase in plasma osmolality has been shown to increase the release of atrial natriuretic peptide in rats (3) but it was not possible to rule out the influence of an increased plasma volume induced by a shift of liquid from the extracellular space. Experiments on isolated rat atria have shown a release of atrial natriuretic peptide induced by hyperosmolality (4). However, the preparation failed to exhibit a normal release of peptide to stretch.

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Isolated atrial cells have also shown an increased release of atrial natriuretic peptide when perifused with hyperosmolar solutions (5) but the susceptibility of isolated cell preparations to external factors, including osmolality, needs to borne in mind.

In view of these doubts, we have examined the effects of temperature and osmolality on the release of atrial natriuretic peptide in an isolated rat atrial preparation which responds to stretch (1). Since an increased temperature inevitably causes an increased frequency of contraction, we have examined, separately, the effects of an increased frequency. The interest of the effect of temperature lies in the insight it may give into the energetics of release of the peptide and, for this purpose, we have studied the effects of a 10°C variation in temperature. The interest of the effects of osmolality, on the other hand, lies in their physiological implications and, for this purpose, we have limited our studies to the physiological range.

MATERIALS AND METHODS

Male Sprague Dawley rats weighing 200-300 g were killed by cervical dislocation. Their hearts were immediately removed and the left and right atria with the septum dissected from the ventricle and placed in Krebs-Henseleit solution (pH 7.4) equilibrated with 95% oxygen and 5% carbon dioxide and maintained at 4°C. The combined atria were then mounted in a polyethylene organ bath of 1 ml volume and superfused at the rate of 1 ml/min with the oxygenated Krebs-Henseleit solution.

Effects of temperature

Two groups, each of 6 atrial preparations, were maintained at 27°C and 37°C, respectively. For these experiments the atria were connected by a thread with a force-displacement strain gauge transducer (Basile Recording Microdynamometer 7050) and pre-loaded with 0.5 g. The atria were beating spontaneously throughout the whole experiment. After an equilibration period of 60 min, the load was increased by 0.5 g for 20 min. The effluent from the bath was collected at 50, 55 and 60 min during the equilibration period and at 1,3,5,8,10 and 20 min following the increment in load.

Effects of increased frequency of contraction

For this purpose 5 atria, subjected to a load of 0.5 g, were paced from the basal rate of 187 ± 6 (S.E.) to 300 beats/min for 30 minutes. The stimulation was then stopped and the effects of stretch demonstrated after 10 minutes by increasing the load from 0.5 to 1.0 g. Electrical stimulation was achieved using supra-threshold rectangular pulses of 1 ms duration from a Tetronix square wave generator. Threshold was determined by slowly increasing the stimulator voltage until capture occurred. The stimulator voltage was then adjusted to 0.2 V above threshold.

Effects of hyperosmolality

In three groups of experiments, each comprising 6 atrial preparations, the osmolality was increased from 290 to 320 mOsm using glucose (38.85 mM), NaCl (133.26 mM) and KCl (31.92 mM) respectively. No basal load was applied to the atria and the temperature was maintained at 32°C. During the 60 min equilibration period the osmolality was maintained at 290 mOsm.

Osmolality was then increased to 320 mOsm for 6 min, at the end of which time it was returned to 290 mOsm for a further 10 min. The effluent from the bath was collected at 50, 55 and 60 min during the equilibration period, at 2, 4 and 6 min during the period of hyperosmolality and at 2,4,6 and 10 min during the second period of normal osmolality.

Radioimmunoassay

We followed the method previously described (1).

Immunoreactivity to atrial natriuretic peptide was done using ^{125}I- alpha-human atrial natriuretic peptide and rabbit antiserum to alpha-human atrial natriuretic peptide. The antiserum had 100% cross-reactivity with rat atrial peptide (6).

Separation of free from bound radiolabel was achieved using goat anti-rabbit serum. The ^{125}I-peptide and the antisera were obtained from Peninsula Laboratories. The minimal detectable concentration of alpha-human atrial natriuretic peptide was 6 pg/ml.

Data are expressed as means ± SEM of groups of 5-6 separate experiments and stastical analysis was based on unpaired Student's t tests.

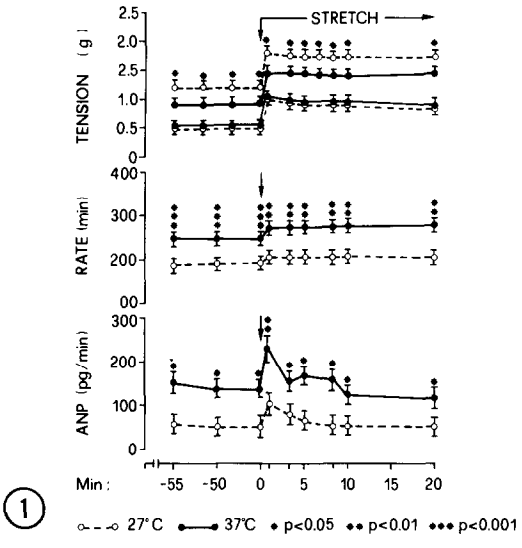
RESULTS

Effects of temperature (figure 1)

The developed force was 40% lower at the higher temperature (37°C) with a load of 0.5 g ($p<0.05$) and 35% lower at the higher temperature with a load of 1.0 g ($p<0.05$). The higher temperature was also associated with an increased frequency of contraction which amounted to 28% at a load of 0.5 g ($p<0.001$) and 30%

immediately after the imposition of a load of 1 g ($p<0.01$). The basal rate of release of atrial natriuretic peptide by the atria superfused at 27°C averaged 76±15 (S.E.) pg/min, while that of the atria superfused at 37°C averaged 154±31 (S.E.) pg/min, yielding a ratio of 2.03.

Stretching the atria at 37°C with an increment of 0.5 g was followed by an increased rate of release of atrial natriuretic peptide, reaching a maximum of 211 ± 27 (S.E.) pg/min after 1 min. The rate of release of the peptide decreased rapidly from then on and, after 10 minutes, had reached a level (121 pg/min) similar to that obtaining before the increment in load. Stretching the atria at 27°C resulted in a small release of atrial natriuretic peptide with a time-course similar to that occurring at 37°C. The total release of atrial natriuretic



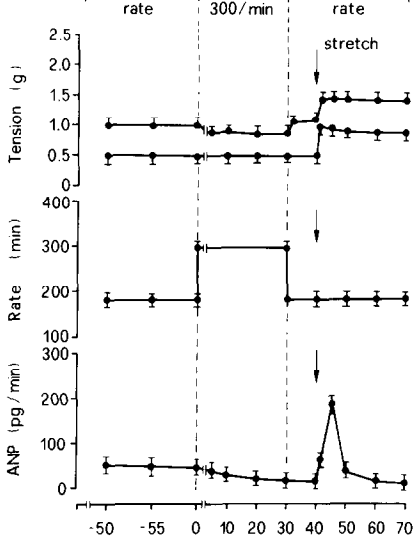


Fig. 1. Influence of increasing temperature (from 27°C to 37°C) on mechanical activity frequency and release of atrial natriuretic peptide by 6 rat atria subjected to stretching. Data are presented as mean ± standard error.

Fig. 2. Effects of inducing an increased frequency of contraction on the release of atrial natriuretic peptide. Data are presented as mean ± standard error.

peptide during the experiment was 2201 ± 87 (S.E.) pg at 27°C and 4654 ± 102 (S.E.) pg at 37°C ($p<0.05$), yielding a ratio of 2.06.

Effects of frequency of contraction (figure 2)

Pacing at 300 beats/min for 30 minutes produced a small decrease in developed force but had no effect on the rate of release of atrial natriuretic peptide. The subsequent increase in load under unpaced conditions resulted in a small decline in force with no change in atrial rate and was followed by a sudden increase in the rate of release of peptide from a basal level of 25 ± 4.9 (S.E.) pg/min to 182 ± 48.1 (S.E.) pg/min after 5 minutes. Thereafter the release of peptide rapidly declined. These experiments showed that an increased rate did not, by itself, cause an increased rate of release of atrial natriuretic peptide and that the preparation was capable of releasing peptide in response to stretch.

The effects of hyperosmolality (figure 3)

Increasing the osmolality with glucose or sodium had no effect on the rate or force of contraction. Increasing the osmolality with potassium, however, temporarily paralysed the atria. There was, clearly, no effect of glucose or sodium on the

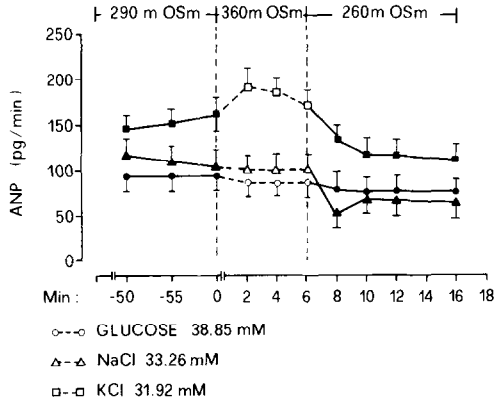


Fig. 3. Effects of increasing osmolality (from 290 to 360 mOSm) on the release of atrial natriuretic peptide. Data are presented as mean ± standard error.

rate of release of atrial natriuretic peptide. Increasing the concentration of potassium appeared to the eye to have been associated with a minute increase in release of peptide in four experiments but this was not apparent in the remaining two experiments and the results, taken as a whole, did not reach a level of statistical significance.

DISCUSSION

The effects of stretch

The transient release of atrial natriuretic peptide in response to a constant increase in load is what we have previously described in the preparation used (1). Its presence, in the present studies, has been taken as an indication of the viability of the tissue.

The effects of temperature

Our results show a distinct effect of temperature on both the basal and the transient stretch-induced release of atrial natriuretic peptide. The finding of a Q_{10} equal approximately to 2 strongly suggests the involvement of an enzymic mechanism in the release of the peptide even if the stimulus for release is mechanical. The course of events from the high molecular weight precursor stored in the atrial granules (7,8) to the ultimate release from the surface of the myocyte of a low molecular-weight peptide has not been clearly charted, although a protease is known to be responsible for the cleavage of the precursor (9,10).

Although a higher temperature is associated with an increased frequency of contraction, our experiments show that does not account for or even contribute to the increased release of peptide. In this respect the results of our experiments are not in conflict with those of Bilder et al (2) in which the atria

were not paced but were, instead, subjected to repetitive stretching at a constant rate.

The effects of hyperosmolality

In our experiments, an increased osmolality within the physiological range and capable of inducing the release of vasopressin (11,12) had no effect on the rate of release of atrial natriuretic peptide. The small effect of potassium, although statistically insignificant, may have been the consequence of depolarisation which has been claimed to cause the release of hormones in the hypothalamus (13,14,15).

These results are in contradiction with those of Arjamaa and Vuolteenaho (4) and Gibbs (5). The discrepancy may be due to the disruptive effects of hyperosmolality on cell membranes. The experiments of Gibbs (5) were done on an isolated myocyte preparation in which the cells, subjected to enzimatic digestion, are likely to be more susceptible than normal to osmotic damage.

Those of Arjamara and Vuolteenaho (4) were done on a preparation whose viability may be doubted from its lack of response to stretch.

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