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Influence of intra-osseous infusion of a small volume of hyperosmotic fluid on β-adrenergic function in circulating lymphocytes from bled pigs

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The regulation of β-adrenergic function in circulating mononuclear leukocytes was evaluated during an episode of haemorrhagic shock treated by intra-osseous infusion of a small volume of hyperosmotic fluid. Two groups of piglets (n = 14) were anaesthetized with ketamine and bled to a mean arterial pressure of 40 mmHg. After 45 min the animals were randomized to receive 100 ml of either hyperosmotic (2.4 mol l^{-1}) or iso-osmotic (0.29 mol l^{-1}) fluid (equal volumes of glucose/sodium chloride) into the tibial bone marrow. Observations of haemodynamic variables and levels of plasma catecholamines and cAMP of circulating mononuclear leukocytes were carried out for 70 min. Infusion of hyperosmotic fluid enhanced the circulatory performance and attenuated the plasma catecholamine release significantly ($p < 0.05$) compared to the corresponding values in the animals that had iso-osmotic fluid infusion. Measurements of unstimulated and isoprenaline-stimulated cAMP levels in mononuclear lymphocytes indicated that the high plasma catecholamine levels in the iso-osmotic treatment group induced a desensitization of the β-adrenoceptors 70 min after initiation of the shock. This effect was not seen in animals that had undergone hyperosmotic infusion.

Key words: β-adrenoceptors; cAMP; catecholamines; haemorrhagic; mononuclear leukocytes; resuscitation

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Infusion of a small volume of hyperosmotic fluid effectively restores the circulation in bled pigs [1]. Such resuscitation may be given by intra-osseous infusion when standard venous access is not accomplished [2]. It has further been observed that the renal clearance of catecholamines is restored during haemorrhagic hypoperfusion [3], and that hyperosmotic fluid infusion rapidly attenuates the increased plasma catecholamine levels in bled pigs [4].

The change in plasma catecholamine levels may produce a different influence of these hormones on the specific cellular receptors [5–11].

Lymphocyte β-adrenoceptors are found to reflect changes of β-adrenoceptors of less access-

ible tissues, e.g. human lung, saphenous vein, and heart (12–14).

The aim of the present study was to determine whether the different levels of plasma catecholamines after hyperosmotic and iso-osmotic infusion can be reflected by effects on the β-adrenergic responsiveness in circulating mononuclear leukocytes.

MATERIAL AND METHODS

Outline of study

A total of 14 piglets were used in these experiments. After stabilization for 1 h after surgical procedures, all animals were bled to a mean arterial pressure of 40 mmHg, which was maintained for 45 min. Resuscitation was then given over a 5-min period with a small volume of either hyperosmotic (n = 7) or iso-osmotic fluid (n = 7) into the tibial marrow. All animals were studied until 70 min after the start of the bleed.

Animals and preparation

A total of 14 male and female Norwegian landrace piglets that weighed between 18.6 and 28.4 kg were used. The animals were fasted for 20 h before the experiment, but had free access to water.

General anaesthesia was induced with an intramuscular injection of ketamine, 500 mg, and atropine sulphate, 1 mg. After intubation the animals were ventilated with equal parts of oxygen and nitrous oxide, controlled by a Servo ventilator (Elema Schönander 900, Stockholm, Sweden). Ketamine was continued as an intravenous infusion of 20 mg kg^{-1} h^{-1} during the whole experiment, and additional injections of fentanyl 0.1 mg × 3 and midazolam 5 mg × 3 were given during the surgical procedures. Sodium chloride, 0.9%, with glucose, 5%, was given through an ear vein cannula (20 ml kg^{-1} h^{-1}) for the 1st h, followed by 10 ml kg^{-1} h^{-1}, using a volumetric pump (IVAC, San Diego, California, USA).

A 5 F Swan–Ganz catheter (Edwards Laboratories, Santa Ana, California, USA) was inserted into the pulmonary artery through the right external jugular vein. A 7 F Swan–Ganz catheter was introduced into the left femoral artery and advanced into the descending aorta. A Foley catheter was placed in the urinary bladder through a cystotomy. All operations and wound closures were done under aseptic conditions.

Preparation of the hyperosmotic and iso-osmotic fluids

The hyperosmotic fluid was prepared by mixing 50 ml of 50% glucose with 50 ml of 1 mol l^{-1} sodium chloride. The osmotic concentration of the fluid was equal to 2.400 mmol l^{-1}. The iso-osmotic fluid (290 mmol l^{-1}) was prepared in the same manner from 5% glucose and 0.9% sodium chloride. Each solution was suspended in a 100-ml infusion bag.

Induction of shock

The haemorrhagic shock was induced after a period of stabilization of 1 h after the instrumentation. Unrestricted bleeding to a mean arterial pressure of 40 mmHg was induced in all animals through a sampling catheter with the tip placed in the superior vena cava. Minor bleeds to stabilize the shock were ended 15 min after induction, and the shock was continued for another 30 min until the animals were resuscitated.

Hyperosmotic and iso-osmotic resuscitation

All animals received 100 ml of either the hyperosmotic or the iso-osmotic resuscitation after the bleed. Infusion was achieved with a 2.0 mm sternal marrow needle with a Luer lock attached which was forced through the upper anteromedial surface of the tibial shaft. Gravity flow was accomplished by hanging the infusion bags 100 cm above the heart. The infusion lasted for 5 ± 1 (mean ± SD) min.

The animals were observed for 25 min after start of the resuscitation before being sacrificed with a lethal dose of pentobarbitone.

Blood samples

Blood samples to prepare plasma for catecholamine measurements were drawn immediately before induction of the shock and at 5, 10, 15, 25, 35, 45, 50, 55, 60 and 70 min during the experiment. Blood samples for cAMP measurements were drawn immediately before the shock and after 40 and 70 min. The sampling tubes were kept on ice awaiting further processing.

Circulatory variables and electrocardiography

The aortic and pulmonary artery blood pressures were monitored continuously through the Swan–Ganz catheters on a multichannel recorder (Recorder 2800-S, Gould, Cleveland, Ohio, USA). Mean arterial pressure, mean pulmonary artery pressure and central venous pressure were calculated at intervals of 5 min. Cardiac output was measured by thermo dilution with a cardiac output computer (9520 A Computer, Edwards Laboratories), and electrocardiography was monitored continuously.

Preparation of lymphocytes and incubation of cells

Arterial whole blood (15 ml) was sampled in polypropylene tubes with EDTA to a final concentration of 3 mmol l^{-1}. Mononuclear leucocytes were isolated by density gradient centrifugation at 4°C [15] using Lymphoprep® (Nycomed, Oslo, Norway). The cells were aspirated and washed three times with ice-cold incubation buffer. The buffer comprised (mmol l^{-1}): NaCl 122, KCl 4.9, $MgSO_4$ 1.2, $CaCl_2$ 1.3, Na_2HPO_4 15.9, D-glucose 5, pH 7.38 at 37°C. Viability, as determined by the trypan blue exclusion test, was 95–99%.

The cells ($2–4 \times 10^6$ cells in 1 ml) were incubated in the absence or presence of 1 mmol l^{-1} (−)-isoprenaline at 37°C for 6 min (incubations in triplicate). The reaction was terminated by addition of ice-cold trichloracetic acid to achieve a final concentration of 300 mmol l^{-1}, and then neutralized with $CaCO_3$. The mixture was centrifuged and the concentrations of cAMP were determined in the supernatant (duplicates) by RIA, using rabbit antiserum, raised according to Steiner *et al.* [16]. The antiserum employed, tested in our own laboratory, had a specificity and sensitivity virtually identical to that described by others [9]. [^{3}H]-cAMP (specific activity 29.4 ci mmol $^{-1}$) and unlabelled cAMP were obtained from Amersham International (Buckinghamshire, England) and Sigma Chemical (St Louis, Missouri, USA) respectively.

Catecholamine analysis

Arterial blood with heparin (4 IU ml^{-1}), reduced glutathione (4.5 mmol l^{-1}) and EGTA (5 mmol l^{-1}) was kept on ice for 30 min before centrifugation at $1000 \times g$ for 20 min at 4°C. The samples were stored at −80°C while awaiting analysis.

Plasma samples (1–2 ml) were spiked with known concentrations of the internal standard (dihydroxybenzylamine) and an added 1 ml of 2 mmol l^{-1} Tris-EDTA buffer (pH 8.7). The catecholamines were adsorbed on to alumina (10 mg). After aspiration of plasma/buffer, the alumina was washed three times with double-distilled water (1 ml). The catecholamines were eluted [17] from the alumina with a mixture (100 µl) comprising acetic acid (175 mmol l^{-1}), sodium bisulphide (9 mmol l^{-1}) and EDTA (0.7 mmol l^{-1}). The alumina was separated from the aqueous phase by filtration (Microfilter® cartridges for low-volume sample clean-up, Bioanalytical Systems, West Lafayette, Indiana, USA).

The catecholamine levels were determined by high performance liquid chromatography. The setup included: 590 Solvent delivery module, U6K injector, 460 electrochemical detector and M740 data module (Millipore, Waters Chromatogrpahy Division, Milford, Massachusetts, USA). A Clin-Rep® column and eluent (Pharma Vertriebs, Munich, Germany) were employed for the chromatography performed at ambient temperature. Eluent flow was 1.0 ml min^{-1}. No interfering peaks were detected, and the cut-off value for adrenaline and noradrenaline detection in pig plasma was 0.03 $pmol^{-1}$.

Statistical analysis

Statistical analysis was performed with a standard statistical package (Statgraphics, STS, Maryland, USA). The Kruskal–Wallis one-way analysis of variance was used to assess the significance of overall differences between the groups at two levels, before and after the infusions. The Friedman two-way analysis was used to test differences within groups. The Wilcoxon non-parametric comparison between two samples was used to assess the differences between the groups at time points. A probability of less than 0.05 was considered statistically significant. Data are expressed as mean ± SEM.

RESULTS

All animals survived the experiment. The hyperosmotic infusion significantly increased the mean arterial pressure and reduced the heart

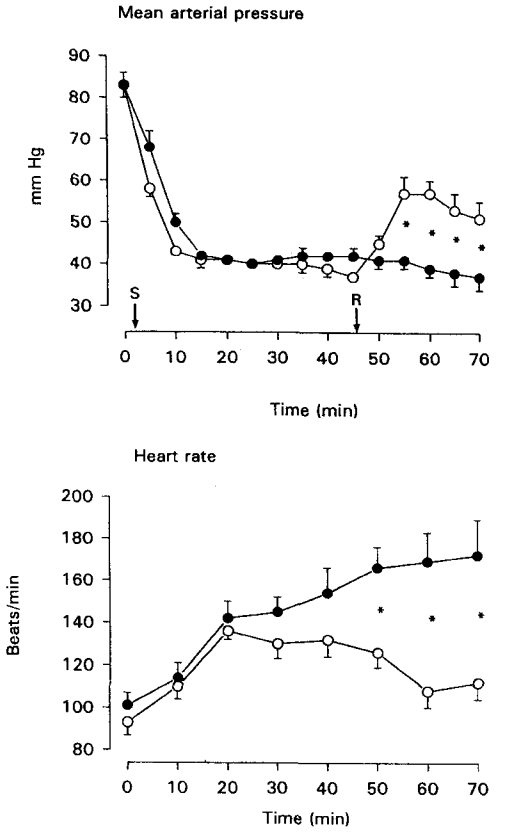

FIG. 1. Mean arterial pressure and heart rate in two groups of animals (n = 7, each group) treated by intra-osseous infusion of either hyperosmotic (open circles) or iso-osmotic (closed circles) fluid (R), after an episode of haemorrhagic shock (S). Differences between the groups at time points are indicated, * $p < 0.05$.

rate compared to the iso-osmotic infusion ($p < 0.05$). Haemodynamic responses are presented in Figure 1 and Table I. At 40 min after the start of the bleed, the plasma adrenaline levels of the hyperosmotic and iso-osmotic treatment groups were increased 5.8- and 4.9-fold, and the plasma noradrenaline levels 8.5- and 9.3-fold compared to the baseline levels, respectively. At 20 min after the hyperosmotic infusion, the plasma adrenaline and noradrenaline levels were reduced to 2.7- and 4.1-fold of the baseline levels, significantly different from the corresponding plasma levels in the iso-osmotic treatment group that were increased to 10.1- and 21.9-fold, respectively ($p < 0.05$). Plasma adrenaline and noradrenaline measurements are shown in Figure 2.

Basal cAMP levels increased by about 40% in both groups after 40 min. At 70 min they were below reference levels in the iso-osmotic treatment group, and significantly lower than those recorded both at 40 min and in the hyperosmotic group at 70 min ($p < 0.05$). In parallel experiments, where the cells were maximally stimulated with isoprenaline, lower cAMP levels (non-significant) were also observed in the iso-osmotic group at 70 min. When the response relative to basal levels was calculated, an increased responsiveness in the iso-osmotic group appeared to exist at 70 min. Unstimulated and stimulated cAMP levels, and cAMP responsiveness are shown in Figure 3.

DISCUSSION

The plasma catecholamine concentrations increased by 5–9-fold compared to baseline

TABLE I. Mean (SEM) measurements of cardiac index, systemic vascular resistance index, and pulmonary vascular resistance index in 14 piglets that were subjected to haemorrhagic shock, and intra-osseous resuscitation by a small volume of either hyperosmotic or iso-osmotic fluid

		Time, min		
Variable	Resuscitation	0 (Shock)	45 (Intra-osseous resuscitation)	70
Cardiac index, $l\ min^{-1}\ m^{-2}$	Hyperosmotic	4.61 (0.21)	1.95 (0.08)	3.15 (0.24)*
	Iso-osmotic	4.58 (0.21)	2.13 (0.08)	1.93 (0.11)
Systemic vascular resistance index, dyne $s\ cm^{-5}\ m^{-2}$	Hyperosmotic	1360 (45)	1644 (74)	1426 (42)*
	Iso-osmotic	1387 (49)	1606 (57)	1611 (64)
Pulmonary vascular resistance index, dyne $s\ cm^{-5}\ m^{-2}$	Hyperosmotic	114 (8)	135 (29)	127 (12)
	Iso-osmotic	95 (11)	108 (6)	142 (21)

Differences at time points between the groups are indicated, *$p < 0.05$.

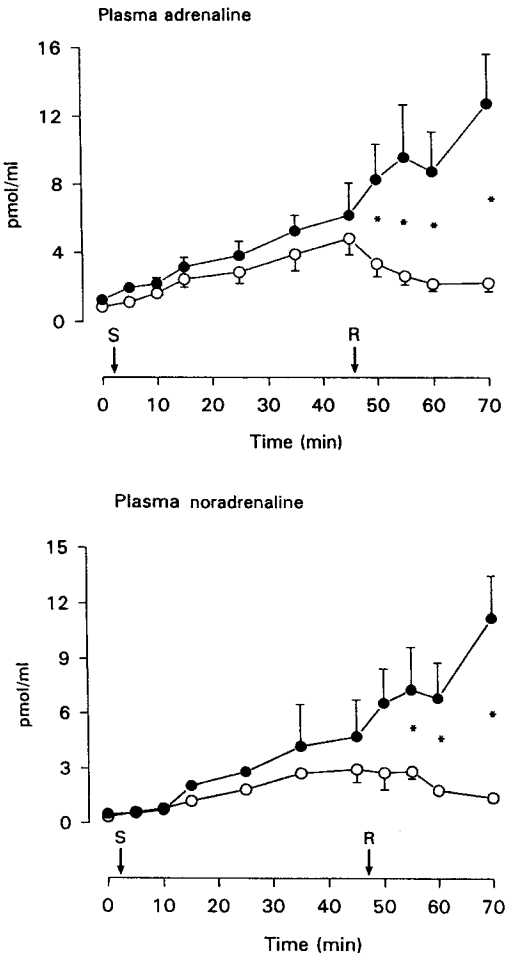

FIG. 2. Plasma adrenaline and plasma noradrenaline in two groups of animals (n = 7, each group) treated by intra-osseous infusion of either hyperosmotic (open circles) or iso-osmotic (closed circles) fluid (R), after an episode of haemorrhagic shock (S). Differences between the groups at time points are indicated, *p < 0.05.

values in both animal groups 45 min after the bleed. Intra-osseous hyperosmotic small-volume infusion increased the mean arterial pressure and cardiac index and reduced the heart rate significantly, compared to an equal volume of iso-osmotic infusion. The plasma catecholamine levels were also reduced after the hyperosmotic infusion, and contrasted significantly with those in the animals that had had iso-osmotic infusion.

The mononuclear leukocytes were prepared at 4°C to maintain the cells in the potentially desensitized state, i.e. receptors internalized

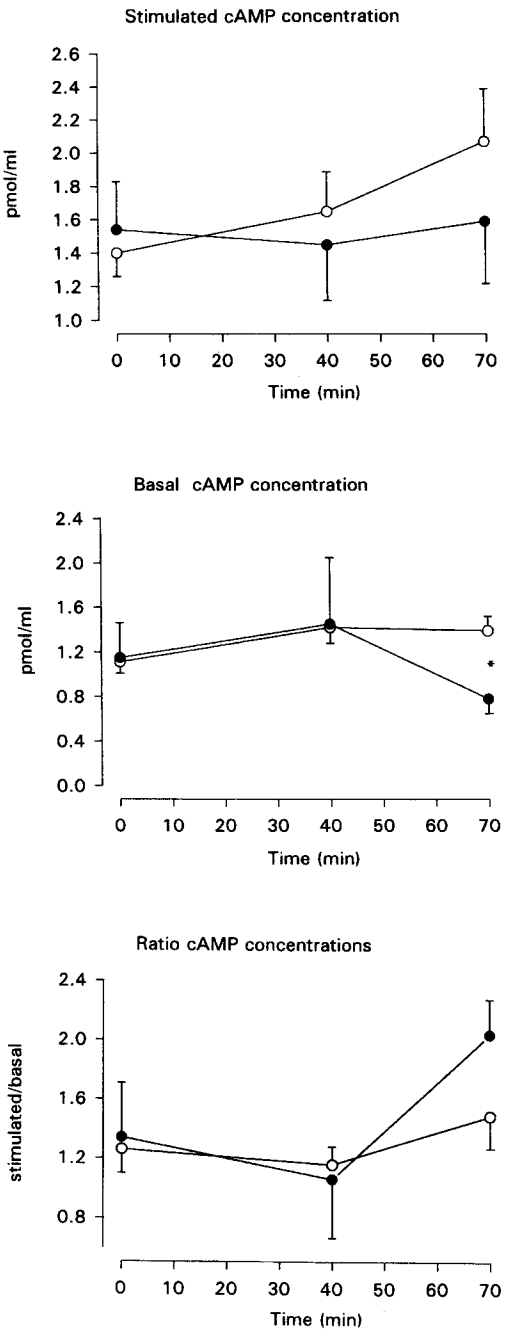

FIG. 3. Stimulated cAMP, basal (unstimulated) cAMP, and ratio between stimulated and basal cAMP in two groups of animals (n = 7, each group) that were treated by intra-osseous infusion of either hyperosmotic (open circles) or iso-osmotic (closed circles) fluid (R) after an episode of haemorrhagic shock (S). Differences between the groups at time points are indicated, * p < 0.05.

and/or uncoupled [15]. This methodological approach may have prevented an efficient catecholamine washout and may explain why the isoprenaline-unstimulated levels increased in both series from 0 to 40 min. In agreement, the catecholamine levels after hyperosmotic resuscitation are reflected in unchanged isoprenaline-unstimulated levels. In contrast, a desensitization of the β-adrenoceptors on the circulating mononuclear leukocytes from the iso-osmotic treatment group was observed. Despite the immense increase of catecholamine levels, the isoprenaline-unstimulated levels fell significantly. The decreased cAMP levels indicate a desensitization [18]. One possible explanation is a reduced interaction between the α_s-subunit of the G-protein and adenylate cyclase.

In parallel experiments, the mononuclear leukocytes were maximally stimulated with isoproterenol. At the start of this experiment, and 40 min thereafter, the cAMP levels were similar in the iso-osmotic and hyperosmotic resuscitation groups, with levels about 50% above isoprenaline-unstimulated levels. At 20 min after the hyperosmotic and iso-osmotic infusions, the cAMP levels tended to increase and decrease, respectively. However, when calculating the isoprenaline-mediated response, a distinctly different pattern was observed between 40 and 70 min. The increase in responsiveness was much more pronounced in the iso-osmotic group, which may suggest that the non-internalized β-adrenoceptors (receptors remaining on the cell surface) were brought to a high affinity (tight coupling) state [8, 18–20].

It has been shown by van Tits *et al.* [21] that an increase in the plasma catecholamine levels may apparently increase the number of β-adrenoceptors by recruitment of lymphocytes from the spleen. However, the present results indicate that the density of β-adrenoceptors was reduced, not increased.

The reduced β-adrenoceptor sensitivity in the iso-osmotic treatment group may be estimated from the haemodynamic parameters. In the period from 40 to 70 min the mean arterial pressure fell by 7–8%, despite no active blood loss and a rapid increase in the plasma catecholamine levels. The heart rate increased by only 10%, to 172 beats min^{-1}, and the cardiac index was reduced by 10%. Interestingly, the systemic vascular resistance index in the iso-osmotic treatment group increased by only 8% during the experiment, as the pulmonary vascular resistance index increased by about 45%, reflecting the major differences between the pulmonary and the systemic vascular beds with respect to receptor quality, density and responsiveness [22].

The physiological and pharmacological consequences of the observed alterations in β-adrenoceptor function were discussed in part by Marty *et al.* [23].

In conclusion, we suggest that the altered β-adrenergic responsiveness in severe bleed can be prevented by the favourable cardiovascular responses after intra-osseous infusion of hyperosmotic fluid. The mechanism behind the change in β-adrenergic tissue sensitivity is not clear, and radioligand binding studies are needed to determine how receptor internalization and receptor coupling are involved.

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