Hypothalamo–pituitary–adrenal axis response to coronary artery embolization: an ovine model of acute myocardial infarction

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Abstract

Although previous studies have described the hypothalamo–pituitary–adrenal (HPA) response to the stress of acute myocardial infarction, it is not possible to study the hormone changes immediately after infarction in humans. Accordingly, we have examined the HPA response to microembolization of coronary arteries in 13 sheep compared with 5 sham control sheep. Plasma vasopressin (AVP; \(P<0.001\)), ACTH \((P=0.005)\) and cortisol \((P=0.005)\) were all increased 2 h (first sample time) after embolization. Plasma ACTH and cortisol levels returned to baseline levels by 6 h but plasma AVP levels did not return to baseline levels until more than 12 h after embolization. Plasma corticotrophin-releasing hormone (CRH) showed no significant change in response to embolization. In a subset of six animals which were sampled more frequently, the peak responses for plasma AVP, ACTH and cortisol occurred at 40 min after embolization. The maximum responses in any individual sheep observed at this time point were 744 pmol/l for AVP, 144 pmol/l for ACTH and 492 nmol/l for cortisol. CRH levels tended to increase across the first hour but these changes were not statistically significant. In conclusion, the stress hormone responses to microembolization of the coronary arteries have been defined in an ovine model of myocardial infarction. This model is suitable for studying the effects of novel treatments to reduce the stress of myocardial infarction.


Introduction

The stress hormones cortisol (Bailey et al. 1967, Jensen et al. 1988) and vasopressin (AVP) (McAlpine et al. 1988, Rouleau et al. 1993) increase in peripheral plasma following myocardial infarction. AVP and corticotrophin-releasing hormone (CRH) act synergistically to stimulate corticotrophin (ACTH) release from the pituitary gland (Gillies et al. 1982, Rivier & Vale 1983, Milsom et al. 1985) and in turn cortisol secretion by the adrenals. In a previous paper we described CRH, AVP, ACTH and cortisol response to the stress of acute myocardial infarction in humans (Donald et al. 1994). Highly significant increases in plasma CRH, AVP and cortisol were observed which fell to within normal limits during the 72 h observation period. However, contrary to expectation, plasma ACTH was low initially and then rose. As there was a delay of up to 6 h between the onset of chest pain and admission to hospital, it was hypothesized that ACTH had initially increased in response to the rise in plasma CRH and AVP, and then been suppressed by the rise in cortisol. It was assumed that this negative feedback effect would have operated mainly at the pituitary level, otherwise CRH and AVP would also have been suppressed. Indeed the plasma AVP levels were so high in some individuals that tissue perfusion may have been further compromised (Crum et al. 1990, Hader et al. 1990). Because of the delay in presentation, even higher AVP levels may have occurred before admission to hospital. An accurate knowledge of the dynamics of the response is important if treatment, such as with an AVP antagonist, is to be administered effectively. It is not possible to study hormone changes immediately after infarction in humans, and so a minimally invasive animal model was employed. In this paper we report the acute changes in the above mentioned stress hormones following coronary embolization in anaesthetized sheep.

Materials and Methods

Studies were performed in a total of 18 Coopworth ewes. Sheep were housed in an air-conditioned light-controlled room and received a standard diet of sheep nuts and chaff providing a daily intake of 40 mmol sodium and 200 mmol potassium. Animals were held in metabolic crates with free
access to water. At least 2 weeks before the study, the sheep were anaesthetized and underwent left lateral thoracotomy for surgical instrumentation to provide haemodynamic monitoring. The protocol was approved by the Animal Ethics Committee of the Christchurch School of Medicine.

Thirteen of the sheep underwent coronary artery embolization under general anaesthesia induced by i.v. thiopentone (15 mg/kg) and maintained with a mixture of halothane, oxygen and nitrous oxide. Through access provided by a 7 French sheath (Cook Co., Miami, FL, USA) placed in a carotid artery, a 7 French left Amplatz I coronary catheter was introduced for selective entry to the left anterior descending or circumflex branches of the left coronary artery. After confirmation of the position of the catheter by coronary angiography, a 0·5 ml bolus of polystyrene latex microspheres (Polysciences, Warrington, PA, USA) was injected into the coronary branch. Microspheres were suspended in deionized water, with each millilitre of solution containing approximately 4000 particles. The average size of the microspheres was 92·5 μm (range 90·19–94·87 μm). Sheep were extubated within 20 min of embolization and most were standing in their crate within 1 h of embolization. The remaining five sheep (sham controls) underwent an identical procedure except latex microspheres were not administered.

Blood samples were drawn from the conscious animal via a left atrial catheter implanted at the prior thoracotomy. Samples were drawn on the morning immediately before embolization to provide baseline data and then again at 2, 4, 6 and 12 h and days 1, 2, 3, 5 and 7 after embolization. The blood was drawn into EDTA tubes on ice, centrifuged at 4 °C, then stored at −20 °C until analysed. Plasma ACTH was measured by RIA (Donald 1977) after extraction using silicic acid (Mallinckrodt, St Louis, MO, USA, 100 mesh) with a recovery of 76%. The detection limit of the assay was 0·5 pmol/l and the intra-assay coefficient of variation was 9·9%. Plasma AVP was measured by RIA (Sadler et al. 1983) after extraction by acetonitrile precipitation with a recovery of 98%. The detection limit of the assay was 0·3% and the intra-assay coefficient of variation was 3·5 pmol/l. Plasma CRH was measured by RIA (Ellis et al. 1990) after extraction by methanol precipitation with a recovery of 83%. The detection limit of the assay was 0·17 pmol/l and the intra-assay coefficient of variation was 5·2%. Cortisol was measured by ELISA (Lewis et al. 1992). The assay had a detection limit of 14 pmol/l and the intra-assay coefficient of variation was 6·3%. For each hormone, all samples from each animal were measured in the same assay to avoid interassay variability.

A subset of six embolized sheep underwent more frequent blood sampling. In addition to the times stated above, samples were drawn on induction of anaesthesia (−30 min), immediately before embolization (0 min) and then at 20 min intervals for 2 h after embolization and 40 min intervals between 2 and 4 h after embolization. Samples were also drawn from the five sham control sheep at these times.

Statistics

As data were not normally distributed, all results were log-transformed before analysis. ANOVA with repeated measures was used to assess changes with time across embolization or sham controls. Where significant changes were observed with ANOVA, a priori Fisher's protected least significant difference tests were performed to determine individual time points that were significantly different from either pre-anaesthetic baseline values (time point −60 min) to assess the effects of anaesthesia or from anaesthetic baseline values (time point 0) to assess the effects of embolization or sham procedure. Data were subsequently back-logged, thus all results are expressed as geometric means ± s.e.m. Statistical significance was assumed when $P<0·05$.

Results

Experiments were completed without mishap and data collection was completed. Coronary artery embolizations all resulted in myocardial infarctions as judged by electrocardiogram changes and rises in plasma creatine kinase and troponin T (Ikram et al. 1996). Haemodynamic changes and the response of plasma atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and plasma renin activity (PRA) are reported elsewhere (Ikram et al. 1996). Briefly, embolization resulted in falls in left ventricular systolic pressure, and cardiac output with rises in left atrial pressure and heart rate. Plasma ANP, BNP and PRA were all significantly raised following embolization.

The results from the 13 sheep that underwent embolization (day 0–7) for the hypothalamo-pituitary–adrenal axis hormones are shown in Fig. 1. Plasma AVP ($P<0·001$), ACTH ($P<0·005$) and cortisol ($P<0·005$) were all increased 2 h (first sample time) after embolization. Plasma ACTH and cortisol levels had returned to baseline levels by 6 h after embolization but plasma AVP levels did not return to baseline levels until after 12 h. The distribution of results was skewed with exaggerated responses observed in a few sheep. Peak levels measured in the 13 sheep occurred at 2 h after embolization. The maximum responses observed were 230 pmol/l for AVP, 144 pmol/l for ACTH and 384 nmol/l for cortisol. As these peaks occurred at the first sampling point after embolization, hence the subset of six sheep which were sampled more frequently. Plasma CRH showed no significant change in response to embolization.

The results from the subset of six embolized animals that were sampled more frequently and the five sham controls are shown in Fig. 2. Plasma cortisol levels were increased
by both the sham operation ($P=0.009$) and embolization ($P<0.001$). However, plasma cortisol levels in the embolized sheep were significantly higher than time-matched sham control data at 20, 40 and 60 min after embolization. Plasma ACTH levels were significantly increased in the embolized sheep ($P<0.001$). Anaesthesia per se had no significant effect on plasma ACTH levels (no significant change from $-60$ min to time 0) but embolization increased plasma ACTH levels within 20 min and levels remained elevated above baseline levels (time 0) until 120 min after embolization. By contrast, plasma ACTH levels were not significantly changed in the sham control group. Plasma AVP levels were significantly increased in both the embolized ($P<0.001$) and sham control group ($P<0.001$). In the sham control group, plasma AVP levels were increased in response to anaesthesia (levels at time 0 significantly higher than those at $-60$ min) but thereafter they did not increase and eventually fell towards pre-anaesthetic levels. Plasma AVP levels were also increased by anaesthesia in the embolization group (levels at time 0 significantly higher than those at $-60$ min). However, unlike the sham control group, plasma AVP levels increased further in response to embolization with levels being significantly elevated above baseline (time 0) at 40 min after embolization. Plasma CRH levels did not change significantly in either the embolized or sham control groups.

Mean responses observed at 2 h (120 min) in the embolized sheep were similar to those observed for all 13 embolized sheep. However, the peak responses for plasma AVP, ACTH and cortisol occurred at 40 min after embolization. There was much variability in the
magnitude of the response to embolization (hence the log transformation), with the maximum levels attained in any individual sheep after embolization being 744 pmol/l for AVP, 144 pmol/l for ACTH and 492 nmol/l for cortisol.

Discussion

After coronary embolization in sheep there is a highly significant rise in plasma AVP which is maximal at 40 min. The magnitude of the mean maximum response is comparable with levels seen in humans on admission to hospital with myocardial infarction (Donald et al. 1994); however, the AVP levels fell less rapidly in humans. A trend for CRH to rise in sheep was not significant, perhaps because of the relatively small number studied and intersubject variability, whereas it was highly statistically significant in humans. There was a marked rise in plasma cortisol in the sheep, but direct comparison with the response in humans is not possible, because of the difference in cortisol-binding globulin levels in the two species.

Differences in the magnitude and duration of stress hormone responses between sheep and human may be due to several factors. Species differences and the extent of myocardial damage may be relevant. In addition, at the moment of infarction the sheep were anaesthetized, whereas the humans were not. Thus it is likely that the pain of infarction was less in the sheep, at least initially, although ischaemic pain in itself does not activate the hypothalamic–pituitary axis in humans unless infarction has also occurred (Bain et al. 1989). The anaesthetic per se or the associated handling was associated with a small rise in AVP as seen in the sham control animals. However, there was no further increase in plasma AVP levels after the anaesthetic baseline (time point 0). Plasma cortisol levels were also significantly increased in sham control animals, this time increasing further from the anaesthetic baseline levels, but this rise was very much less than that which followed embolization. However, we were less concerned with the magnitude of the hormonal responses than with the temporal relationship between peaks of hormone secretion.

In the sheep the peaks of AVP, ACTH and cortisol secretion were synchronous. In humans studied over 72 h commencing within 6 h of the onset of chest pain, the concentrations of AVP, cortisol and CRH fell, while ACTH concentrations rose. The results in the sheep suggest that AVP and cortisol levels in the humans may have been higher had they been studied earlier, and that ACTH levels may have been raised initially following infarction. However, no comparable secondary fall then rise in ACTH could be demonstrated in the sheep. Myocardial infarction in humans is probably a more complex stressful stimulus than in sheep, involving the fear of dying, the stress of an ambulance ride, the commonplace administration of aspirin, β-blockers, opiates, oxygen and thrombolytics and the unfamiliar surroundings of a coronary care unit. It is difficult to mimic all these possibilities with an animal model.

Peak levels of AVP as high as 744 pmol/l were seen in the sheep. Plasma levels of less than 300 pmol/l are sufficient to cause cutaneous vasoconstriction in man (Hader et al. 1990). Administration of AVP to conscious rabbits results in a rise in total peripheral resistance, and a fall in heart rate and cardiac output with plasma levels in the region of 200 pmol/l (Elliott et al. 1985). The sensitivity of blood vessels in different organs to vasoconstriction by AVP differs, but constriction of coronary arteries and reduced coronary blood flow have been reported (Heyndrickx et al. 1976). Following myocardial infarction, a severe vasoconstrictor abnormality involving not only resistance vessels in infarcted myocardium, but also those in myocardium perfused by normal coronary vessels occurs (Uren et al. 1994). Myocardial viability appears to be associated with the presence of collateral blood flow within the infarct bed (Sabia et al. 1992). Blockade of the plasma catecholamine (The Norwegian Multicenter Study Group 1981) and angiotensin (Pfeffer et al. 1992, Foy et al. 1994, GISSI–3 1994, ISIS–4 1995) responses to myocardial infarction in humans has been shown to improve patient survival. It is clear from this study in sheep that plasma AVP rises almost immediately with onset of infarction, and if AVP V1 receptor blockade is to be considered as a means of improving tissue perfusion, treatment may best be administered early. For maximum effect administration within 40 min would be desirable, although plasma AVP levels did not return to baseline for 24 h in the sheep and human studies. It remains to be seen whether this would have a beneficial effect, with reductions in coronary resistance and improved coronary flow counterbalancing the overall reduction in coronary perfusion pressure secondary to any fall in systemic arterial pressure. It is also not clear to what extent V1 receptor antagonists would block V3 receptors and hence the ACTH and cortisol responses to myocardial infarction. However, a selection of AVP receptor antagonists is now becoming available with varying affinities for these two receptors (Ellis et al. 1994). Hence selective V1 receptor blockade should be possible.

In conclusion, microembolization of the coronary arteries provides a model of myocardial infarction in the sheep. The stress hormone responses have been defined, and hence the effects of treatment in reducing the stress of myocardial infarction can be assessed. Further studies are in progress to determine whether V1 receptor antagonists will improve perfusion and reduce myocardial damage.

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References


GISSI-3 (Gruppo Italiano per lo Studio della Sopravvivenza nell’Infarto Miocardico) 1994 Effects of lisinopril and transdermal glyceryl trinitrate singly and together on 6-week mortality and ventricular function after acute myocardial infarction. Lancet 345 1115–1122.


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