Arterial and venous plasma concentrations of adenosine during haemorrhage

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A haemorrhage model was used to impose severe metabolic stress in anaesthetized cats by removing blood (15.3 ml min^{-1}) to attain an arterial pressure of ca. 50 mmHg for a 2 h period. Adenosine levels in central venous blood rose by 5 min, reached a peak of about 3.5 times control levels by 15 min and then returned to the basal level ($1 \mu\text{M}$) by 60 min. However, the adenosine concentration in arterial blood remained unchanged for the entire 2 h period of hypotension. These data demonstrate that haemorrhage results in rapid adenosine release, but the released adenosine is not able to serve a role as a systemic circulating vasodilator even in this severe model.

Keywords: Adenosine; haemorrhage; anaesthetized cats

Introduction Extreme metabolic stresses, such as ischaemia and haemorrhage, evoke endogenous adenosine release. The released adenosine dilates vascular smooth muscles and restores tissue blood flow to exert protective effects including limitation of vascular injury (Babbitt *et al.*, 1989), improvement of myocardial recovery (Bolling *et al.*, 1990) and prevention of neuronal damage (Dux *et al.*, 1990). Adenosine receptor antagonists, caffeine in rats (Conlay *et al.*, 1988) and 8-phenyltheophylline in cats (Lutt & Légaré, 1985), result in less decrease of blood pressure for a given volume of blood loss. It is unclear, however, if this evidence of a hypotensive role of adenosine is entirely secondary to actions locally at the site of the adenosine release or whether adenosine can recirculate to serve a generalized systemic hypotensive effect. It has been suggested that adenosine release, which is reported to be maximal at the time of haemorrhagic decompensation, may account for the decline in plasma catecholamines that is seen at that time (Tung *et al.*, 1987).

We have examined the relation of endogenous adenosine to haemorrhage using an improved high performance liquid chromatography (h.p.l.c.) fluorometric method for measurement of adenosine in plasma (Zhang *et al.*, 1991). The objective was to determine if adenosine is released into venous blood and, second, if locally released adenosine could attain levels in arterial blood that could lead to generalized systemic effects. The data show that adenosine is released into venous blood during haemorrhage but is eliminated from plasma before reaching the arterial side of the circulation, suggesting that adenosine is not able to serve a role as a systemic circulating vasodilator even under conditions of extreme metabolic stress.

Methods Experiments were performed on mongrel cats (2.5–5.0 kg) of either sex. The animals were fasted for 18 h and anaesthetized with sodium pentobarbitone (32.5 mg kg^{-1} , i.p.); supplementary doses (6 mg, i.v.) were given as required to maintain adequate anaesthesia. Body temperature was maintained at 37.5°C by use of a rectal probe and a thermal-controlled unit (Yellow Springs Instruments, model 72) operating heating rods in the surgical table. A cannula was placed in the right brachial vein for administration of fluids and maintenance doses of anaesthetic. The left femoral artery was cannulated for measurement of systemic arterial pressure, and the aorta was cannulated via the right femoral artery for the withdrawal or infusion of blood. The left carotid artery was cannulated for arterial sampling. The inferior vena cava was cannulated by two catheters via the right and left femoral

vein, respectively. The right catheter, with the tip located below the junction of the renal vein, was used for measurement of central venous pressure and the left one, with the tip located 2 cm below the heart, was used for venous sampling.

Plasma adenosine was measured by h.p.l.c.-fluorometric detection as previously described (Zhang *et al.*, 1991). Briefly, each blood sample ($360 \mu\text{l}$) was rapidly collected into a 1 ml syringe containing $40 \mu\text{l}$ of a stopping solution consisting of dilazep (1 mM) to inhibit adenosine uptake into and release from erythrocytes; erythro-9-(2-hydroxy-3-nonyl)adenine ($10 \mu\text{M}$) to block adenosine deaminase activity and indomethacin ($2 \mu\text{g ml}^{-1}$) to inhibit nucleotide release from platelets (final concentrations). Blood samples were immediately centrifuged, deproteinized with 50% trichloroacetic acid, and neutralized with 3.3 N potassium hydroxide. Adenosine in blood samples was converted by use of chloroacetaldehyde into N⁶-ethenoadenosine for h.p.l.c. analysis.

After the surgery, 4000 units of heparin were administered intravenously via the right brachial vein and each animal was permitted a 1 h stabilization period. Then, four control samples at 0, 15, 30, 60 min before starting haemorrhage were taken for analysis of adenosine and blood gases. The haemorrhage model (Lutt *et al.*, 1980) utilizes a rapid arterial blood withdrawal (15.3 ml min^{-1}) with the rate held steady by a Harvard withdrawal-infusion pump. The animal was bled into a heparinized 60 ml syringe until blood pressure reached 50 mmHg (1 mmHg = 133.322 Pa), which was designated as time 0. At that time haemorrhage ceased and the blood pressure was held at ca. 50 mmHg by withdrawal or infusion of blood for 120 min. Samples of blood were drawn for analysis at 5 min and then at the end of each 15 min period.

Data are expressed as mean $\pm$ s.e.mean. Statistical evaluation was performed by blocked ANOVA. Differences were considered significant at $P < 0.05$.

Results Systematic arterial blood pressure was $121.3 \pm 3.4 \text{ mmHg}$ and during withdrawal of blood the mean pressure decreased to $51.1 \pm 0.7 \text{ mmHg}$ where it was held for 2 h by withdrawal or return of blood. Central venous pressure declined from $4.3 \pm 0.1 \text{ mmHg}$ to $3.2 \pm 0.2 \text{ mmHg}$ by 5 min and remained at a similar level thereafter. The basal levels of arterial and venous plasma adenosine were similar (ca. $1 \mu\text{M}$) and stable within 1 h after surgical preparation (Figure 1). The concentrations of plasma adenosine in central venous efflux were significantly increased from 5 min to 45 min after haemorrhage and then declined back to the basal levels ($P < 0.05$, Figure 1a). In contrast, plasma adenosine concentrations in arterial blood were not elevated during the whole haemorrhage procedure (Figure 1b). Figure 2 represents blood volume removed to cause blood pressure to decline to ca.

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Figure 1 Central venous (a) and arterial (b) plasma adenosine concentrations [Aden] before (open columns) and after (stippled columns) haemorrhage. * denotes that the mean of the post-haemorrhage data is significantly different from the mean of the pooled prehaemorrhage data ($P < 0.05$, blocked ANOVA).

Figure 2 Blood volume removed to maintain blood pressure at the haemorrhage set point of ca. 50 mmHg. The initial rate of bleeding was 15.3 ml min^{-1} until pressure reached the set point whereupon additional blood was withdrawn or replaced as required. All times are measured from time 0, i.e., the time at which blood pressure reached 50 mmHg.

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50 mmHg and be maintained at that pressure. The initial rate of blood removal was rapid (15.3 ml min^{-1}) and was maintained until pressure reached 50 mmHg ($6.4 \pm 1.1 \text{ min}$). The times referred to throughout are taken from $t = 0$ at the time the blood pressure reached the set point. The volume of blood withdrawn reached a peak of $35.3 \pm 4.3 \text{ ml kg}^{-1}$ at 15 min.

Discussion Adenosine is produced and released from a variety of tissues in response to metabolic stress. A wide range of effects on blood vessels, metabolism, nerve functions and hormonal secretion occur in response to local adenosine. However, it is not clear if adenosine is able to serve a hormonal role by attaining sufficiently high circulating levels to cause systemic effects at sites distant from the sites of production. It has been suggested that elevated adenosine levels may suppress catecholamine release (Tung *et al.*, 1987) and lead to decompensation after prolonged haemorrhage in rabbits. Our results do not support this conclusion. First, the pattern of release of adenosine in our results is contradictory to that of Tung *et al.* (1987). Tung *et al.* (1987) found no elevation of venous adenosine 30 min after the onset of haemorrhage and after 1 h the levels were less than $2 \mu\text{M}$ (control levels $0.7 \mu\text{M}$); at the point where 60% of the blood had to be reinfused to maintain arterial pressure at the set-point (41 mmHg) the venous adenosine levels were further elevated to $2.4 \mu\text{M}$. In contrast, our results showed that the peak time of adenosine release correlated well with the peak time of blood volume removed and there was no correlation between adenosine release and decompensation as reported by Tung *et al.* (1987).

Regardless of the differences in release pattern and venous plasma concentration, the possibility of adenosine serving a role as a circulating vasodilator or autonomic nervous modulator is unlikely. Despite the sharp rise in venous levels, the adenosine was eliminated from the blood prior to reaching the arterial side of the circulation. Adenosine is, therefore, not capable of serving a hormonal-type function of producing effects at sites removed from the tissues of production. The possible exceptions would be that even though it was not clear where the adenosine carried in venous blood came from, adenosine may modulate functions downstream in the heart or lungs or in the tissues downstream of the portal circulations of the pituitary, kidney, and liver.

Adenosine appears to contribute to the hypotension seen during haemorrhage since caffeine in rats (Conlay *et al.*, 1988) and 8-phenyltheophylline in cats (Lautt & Légaré, 1985) decreased the hypotensive response. However, the effects are probably restricted entirely to those tissues that generate the adenosine since recirculation of adenosine does not occur. Since such effects are not seen during a major and generalized stimulus like haemorrhage, it seems unlikely that any other localized stimuli would lead to elevations in arterial adenosine levels.

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