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Changes in Inspiratory Activity after Injection of Adenosine and Hypoxanthine in Cats

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ABSTRACT

The effects of intra-arterial and intravenous injections of adenosine and hypoxanthine were investigated with special reference to respiratory variables in anesthetized young cats. Studies were made of the effects on inspiratory activity (phrenic nerve activity), heart rate, blood pressure and central venous pressure. To assess the risk of accumulation of adenosine degeneration products after several injections measurements were also made of hypoxanthine, xanthine and urate in plasma at intervals after the injections. It was found that intra-arterial and intravenous injections of adenosine increased central inspiratory activity during the first few breaths after the injection. The blood pressure and heart rate decreased slightly and central venous pressure increased slightly after the injection. Degradation of adenosine and its metabolites takes place rapidly and it is therefore unlikely that metabolites influence the results. It is concluded that adenosine causes brief stimulation of inspiratory activity.

INTRODUCTION

Hypoxia can lead to a series of reflexes, initiated in an attempt by the body to compensate for the condition. One such reflex is an increased effort to breathe. In addition, hypoxia results in degradation of purine nucleotides and their release into the circulation. Hypoxanthine, which is one of these degradation products, has therefore been utilized to estimate the degree of hypoxia in asphyxiated infants (11,12).

We considered it possible that if purine compounds are released into the circulation in large amounts as a result of hypoxia, they might be involved in the control of breathing. The finding of specific receptors for adenosine (2) supported the idea that this nucleoside might play a role in humoral physiological control mechanisms. Hitherto adenosine has mainly been described as a local defender against hypoxia. It induces vasodilatation (1) and reduces metabolism (2). It has also been reported that injections of adenosine lower the arterial blood pressure, slow the heart rate and reduce body temperature (5), and it may thus have a protective effect on the heart even if such effects may enhance the hypoxia in other organs.

The aim of this study was to determine whether injection of adenosine into the circulation has any influence on breathing in terms of central inspiratory activity (phrenic nerve activity), tidal volume, inspiratory flow, pleural and airway pressures. Adenosine was therefore injected into a central vein during spontaneous breathing and during high frequency positive pressure ventilation, which inhibits spontaneous breathing (4,8). To rule out the possibility of pH effects of the injected solution and possible chemoreceptor stimulation, adenosine and buffer solutions of different pH were injected both intra-arterially and intravenously.

METHODS

Animals and anesthesia

Eighteen cats of both sexes with a body weight of between 2.5 and 5.3 kg were used. The cats were healthy and young but fully grown. Anesthesia was induced with chloroform and maintained with intermittent infusions of chloralose (E.Merck AG, GFR). An endotracheal tube was inserted so that its tip lay about 1 cm above the carina. A flow transducer was attached to the free end of the endotracheal tube. A catheter was introduced into the aorta and another into a central vein through femoral vessels. The tips of both catheters were within the thoracic cage. In addition a catheter was inserted into the endotracheal tube and a further one was introduced into the pleural space through the rib cage. The phrenic nerve was

identified and carefully dissected free from surrounding connective tissue. In some experiments the cats were ventilated by high frequency positive pressure ventilation so that spontaneous respiration was inhibited (8).

Measurements

Arterial and central venous blood pressure and intratracheal and pleural pressures were measured by means of transducers (SE Labs (EMI), Ltd.G.B.) and an amplifier (Elcomatic, EM 760 G.B.) and recorded on a strip chart recorder (Hellige Recorder 330-P). Tidal volume and flow were measured by a pneumotachograph (Mercury CS 5, U.K.). Phrenic nerve activity was recorded by placing the uncut phrenic nerve on two platinum hook electrodes immersed in mineral oil. The signal thus obtained was amplified, filtered and rectified (Neurolog; Digitimer Ltd, G.B.; preamplifier NL103, AC-amplifier NL105, filters NL115, spike trigger NL200) and fed to an integrator. This signal was again amplified (EMMA, SE Labs, Ltd, G.B.) and registered on the same recorder.

Experimental procedure

To rule out the possibility of accumulated effects of consecutive doses, plasma concentrations of hypoxanthine, xanthine and urate were measured. Care was taken to avoid release of purines from erythrocytes and metabolic conversion of purines present in the blood sample. The blood samples were drawn into precooled heparinized tubes containing a xanthine oxidase inhibitor (Allopurinol 100 µl; 1.0 mol/l). After cooling, the samples were centrifuged and plasma was withdrawn, and the plasma proteins were precipitated by addition of perchloric acid (0.75 mol/l; volume ratio 1:1). The purine concentrations were determined by high pressure liquid chromatography. It was found that if injections were made at an interval of 15 minutes or more, the risk of accumulation of the drugs was negligible (Fig. 1).

To investigate whether intra-arterial and intravenous injections of adenosine and hypoxanthine influenced the central inspiratory activity (phrenic nerve activity) and the other recorded variables, adenosine was injected at a concentration of 5 mmol/l and hypoxanthine at 10 mmol/l. The pH of the adenosine

solution was 6.3 and it had no buffer capacity. In these experiments phrenic nerve activity and pressures were recorded before

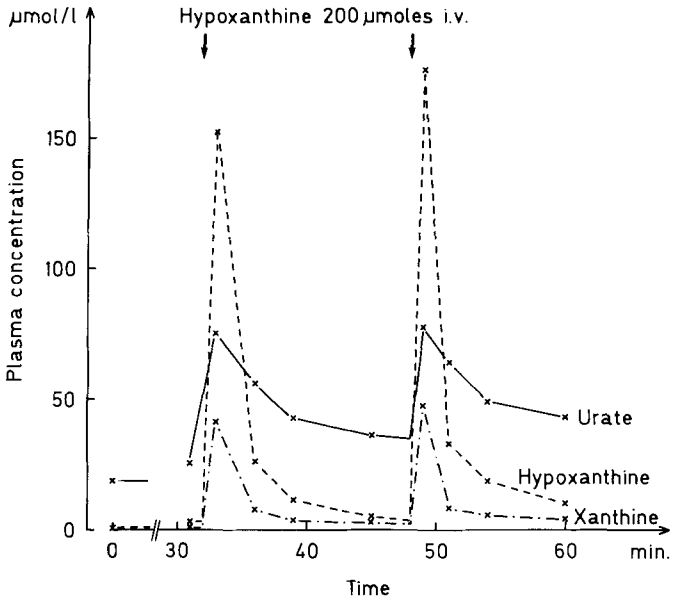

Fig.1 Plasma concentrations of hypoxanthine, xanthine and urate after intravenous injection of hypoxanthine in chloralose-anesthetized cats.

and continuously after intravenous or intra-arterial injection of 20 µmol (4 ml) and 40 µmol (8 ml) of adenosine in spontaneously breathing cats, given a low continuous positive airway pressure to avoid progressive formation of atelectasis. The same procedure was followed when 200 µmol (20 ml) of hypoxanthine was injected. In three cats solutions of adenine and inosine were accidentally injected instead of adenosine and hypoxanthine. In two experiments the spontaneous breathing was inhibited by high frequency ventilation before injection of adenosine or hypoxanthine.

To rule out possible effects of differences in the pH of the injected solutions, the same protocol as above was used in spontaneously breathing cats given a low continuous positive airway pressure. The pH of the solutions were 7.4, 7.0, 7.8 and they were always given in this order. Buffer solutions of the same pH were injected as controls.

Before all experiments and regularly during the experiments arterial blood gases and pH were checked and corrected if necessary.

Treatment of data

The mean impulse frequency in the phrenic nerve was calculated and the intratracheal and intrapleural pressures were measured for five or ten respiratory cycles before and after the injections. The mean impulse frequency was calculated as the quotient of the amplitude and duration of the integrated phrenic nerve recording. Arterial blood pressure and central venous pressure were measured during the same period as that during which the respiratory variables were determined.

Student's t test for paired observations was used in the statistical analysis.

RESULTS

In this study it was found that intravenous injection of adenosine influenced the central inspiratory activity. When adenosine was injected during spontaneous breathing, there was a marked increase in central inspiratory activity during the first two breaths after the injection. After this increase the central inspiratory activity returned to the pre-injection level (Fig. 2). The effect of adenosine was dose-related, the most marked effect in tidal volume being observed after an injection of 8 ml ($p<0.05$; Fig.3). The increase in central inspiratory activity was accompanied by increases in tidal volume, inspiratory flow rate and intrapleural pressure (Fig.2), while the breath interval showed no or only minor changes. Injections of hypoxanthine caused occasional increases in central inspiratory activity, whereas inosine and adenine, which were injected in a few experiments, caused no changes in the measured variables. Injection of adenosine regularly decreased the arterial blood pressure and heart rate.

A marked return of central inspiratory activity was observed when adenosine was injected during high frequency positive pressure ventilation. Control injections of saline did not result in any increase in central inspiratory activity (Fig. 4).

Injection of adenosine into the carotid artery influenced the central inspiratory activity only when the injected solution had a pH that differed from 7.4. Thus injection of adenosine

(20 µmol; 4ml) with a pH of 7.0 and 7.8 into the **carotid artery** increased the central inspiratory activity ($p<0.05$), whereas injection of adenosine with a pH of 7.4 into the carotid artery caused no changes in this activity.

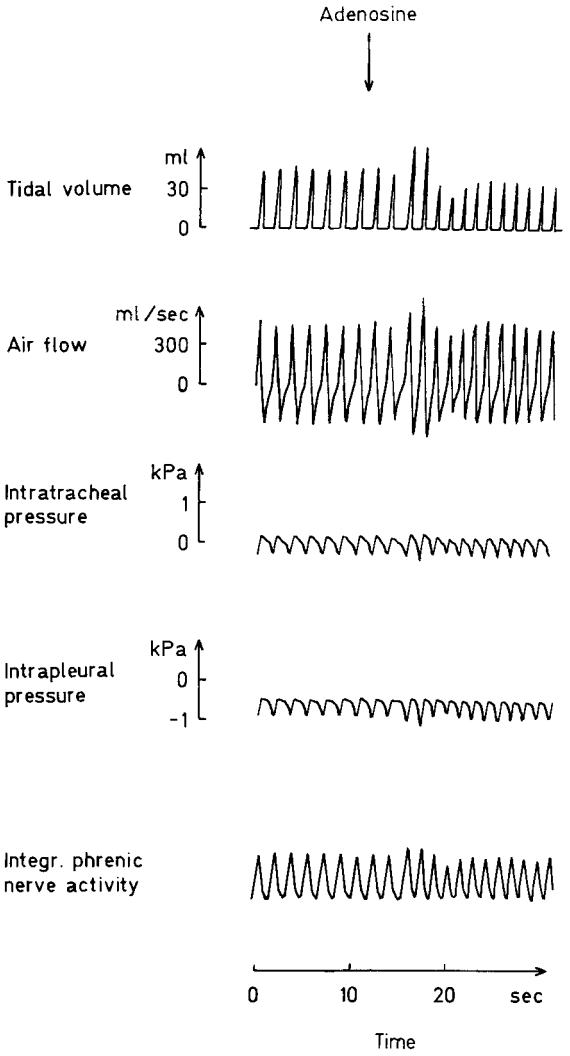

Fig.2 Recordings of tidal volume, air flow rate, intratracheal and intrapleural pressures and integrated phrenic nerve activity in a chloralose-anesthetized cat after intravenous injection of adenosine.

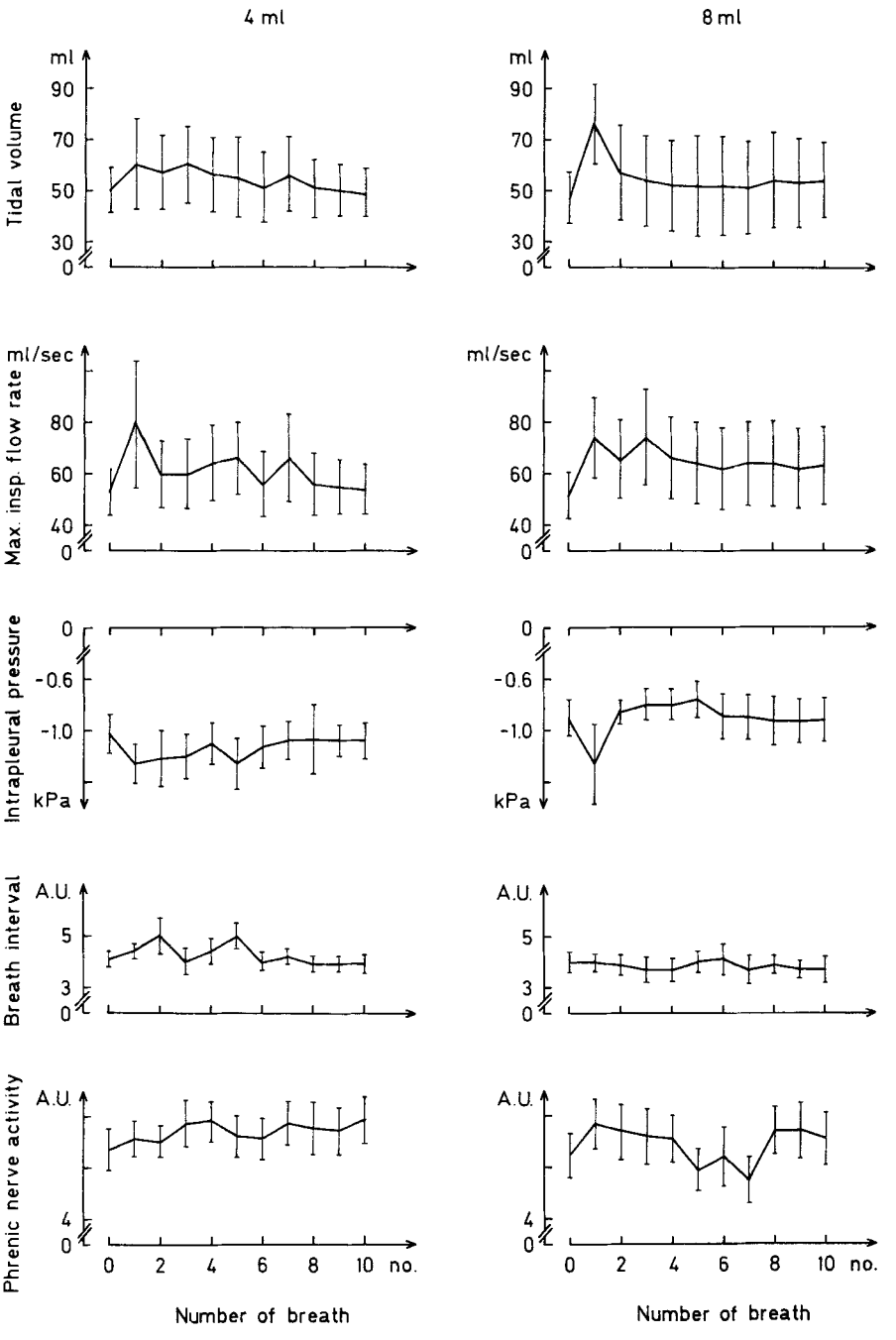

Fig.3 Mean values (± S.E.E.) for tidal volumes, inspiratory flow rate, intrapleural pressure, breath interval and mean inspiratory activity in ten cats after intravenous injection of adenosine.

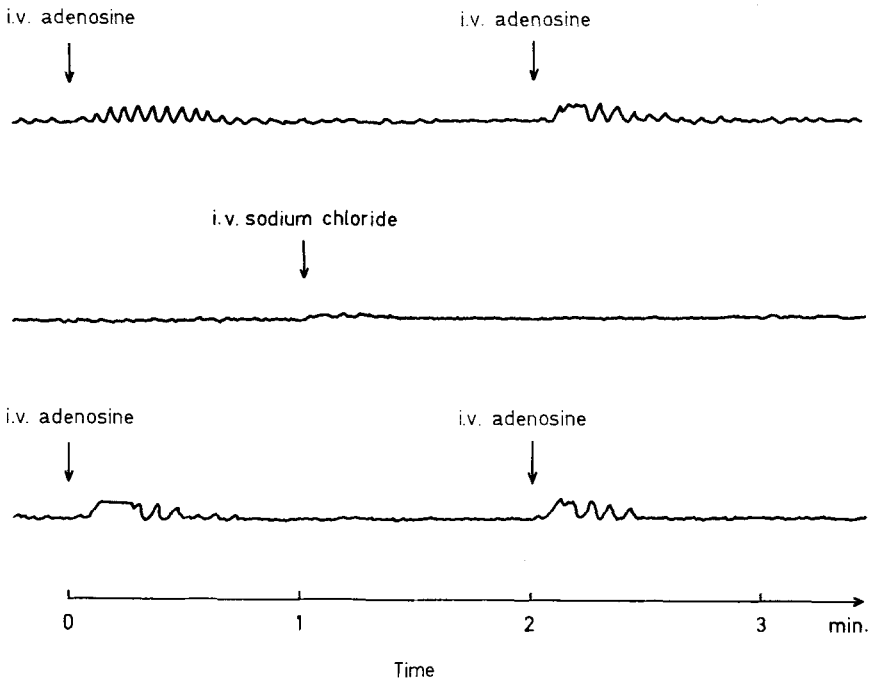

Fig.4 Recording of integrated phrenic nerve activity before, during and after intravenous injection of adenosine in a chloralose-anesthetized cat subjected to high frequency positive pressure ventilation so that spontaneous breathing was inhibited. Injection of adenosine transiently restores the phrenic nerve activity.

Injection of hypoxanthine into the carotid artery had variable effects on the central inspiratory activity. Injection of buffer solutions at pH of 7.0, 7.4 and 7.8 caused no changes in central inspiratory activity. Injection of adenosine into the carotid artery increased the breath interval during the first 5 breaths after the injection of all three adenosine solutions (pH 7.0, 7.4, 7.8), whereas the breath interval remained unaffected after injection of hypoxanthine or buffer solutions into the carotid artery.

Injection of adenosine (20 umol; 4 ml) into a **central vein** caused an increase in central inspiratory activity, which was most pronounced when the injected solution had a pH of 7.8 ($p<0.05$). Hypoxanthine given into a central vein caused no clear changes in inspiratory activity.

DISCUSSION

We found in this study that adenosine increased the central inspiratory activity in fully grown, young cats. The reason for this increase is not clear. It is generally considered that adenosine exerts its effects via specific receptors and that theophylline acts as a competitive antagonist at these receptor sites (2). McQueen & Ribeiro (9), who found an increase in peripheral chemoreceptor activity during infusion of adenosine into the carotid artery in cats, reported that theophylline did not prevent the excitatory action of adenosine on chemoreceptor discharge. On the contrary, the response to adenosine increased after administration of theophylline, suggesting that it has a different influence on, for example sensory nerve endings. McQueen and Ribeiro did not report the pH of their adenosine solution, but at least in some experiments adenosine was dissolved in 0.9% NaCl which gives the solution a low pH. The pH of the adenosine solution might be of importance, as injection of adenosine into the carotid artery caused an increase in the central inspiratory activity in the present study only when the pH differed from 7.4. On the other hand, injection of adenosine solution into the central vein increased the central inspiratory activity irrespective of the pH of the solution. This implies that the mixing of the solution into the larger blood volume eliminates the effect of the pH, which may indicate that metabolic changes of adenosine take place between the site of injection and the carotid chemoreceptors, or that the effect of adenosine injection into a central vein is mediated by stimulation of other receptors, for example in the heart or the pulmonary vasculature. In the present study inadvertent injection of solutions of adenine and inosine instead of adenosine and hypoxanthine had no effect on the central inspiratory activity and serves as a good control of the results reported.

The stimulatory effects of adenosine on central inspiratory activity in the present study are contrary to the findings by several other authors. Thus, this nucleoside has been reported to have an inhibitory effect on respiratory variables when administered into the cerebral ventricles of anesthetized piglets (6) and cats (3). Lagercrantz et al (7) and Runold et al (10) also found that adenosine given intraperitoneally to rabbit

pups depressed breathing - an effect that could be reversed by injection of theophylline. The different sites of administration make stimulation of different receptors possible. Moreover, the metabolic conversion of adenosine may vary with the site of injection. In addition, some of these studies have been made with the animals placed in a body plethysmograph, and it is therefore probable that other actions of adenosine, such as a reduction of the metabolic rate and body temperature (5), might have influenced their results.

From this study we conclude that intravenous injection of adenosine transiently stimulates central inspiratory activity.

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