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Adenosine Analogues Depress Ventilation in Rabbit Neonates. Theophylline Stimulation of Respiration via Adenosine Receptors?

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Summary

The effect of the adenosine analogue L-*N*-phenyl-isopropyl-adenosine (L-PIA) and theophylline on the respiration of rabbit pups was studied. L-PIA (5 μmol/kg) administered intraperitoneally caused a marked respiratory depression in urethane-anaesthetized decerebrate pups and unanaesthetized intact animals during natural sleep. The effect could often be reversed with theophylline. When L-PIA was given after theophylline (20 mg/kg), the effect of L-PIA was considerably lower. L-PIA also caused respiratory depression when administered onto the exposed surface of the fourth ventricle. The effect of the adenosine analogue was more pronounced in younger than in older animals. We conclude that adenosine strongly inhibits respiration and that effect is antagonized by theophylline.

The hypothesis is put forward that the therapeutic effect of theophylline on neonatal apnea might be exerted via adenosine antagonism rather than via inhibition of phosphodiesterase. Apnea in infants is often triggered by hypoxemia. It is possible that adenosine, which is released during hypoxia, mediates this effect.

Abbreviations

CSF, cerebral spinal fluid
L-PIA, L-*N*-phenyl-isopropyladenosine

Methylxanthines are used routinely in the treatment of apnea of preterm infants. The efficacy of both theophylline and caffeine has been documented in a number of studies (1), but the mechanism of action is not known with certainty. Methylxanthines inhibit phosphodiesterase and consequently raise levels of cyclic AMP; however, to achieve this effect, the amount of methylxanthine necessary is considerably higher than the clinically used dosage (6, 10). It has been suggested that methylxanthines antagonize the actions of adenosine at the receptor level (6, 9, 10, 15). Adenosine has been shown to exert clinical depressant actions (5, 9, 15, 16). Furthermore, adenosine has been found to be released during hypoxia (9, 11, 15, 17, 20), which is well-known to cause apnea and respiratory depression in preterm infants (2, 12). The aim was to study the effect of an adenosine receptor agonist on the respiration of rabbit pups.

L-PIA, a stable adenosine receptor agonist analogue, was used in these experiments which were carried out on decerebrate, anaesthetized, or sleeping animals. The antagonism of the L-PIA could be prevented or reversed by theophylline. Preliminary reports on the effect of adenosine analogues on the respiration of newborn piglets and rabbit pups have been published by another group and us, respectively (8, 19) in abstract form.

MATERIAL AND METHODS

Preparation. Nineteen rabbit pups reared by does until the day of experiment were studied between 1–23 d of age. Eleven of these animals were anaesthetized with intraperitoneal injection of urethane in doses of 1.0–1.5 g/kg body weight. Five animals were decerebrate at an intercollicular level under ether anaesthesia. Three unanaesthetized animals were studied in natural sleep. The experimental protocols are summarized in Table 1.

Recordings. The anaesthetized and decerebrate animals were tracheostomized and the respiration was monitored with a small pneumothachograph, which was connected to the tracheal cannula. Tidal volume records were obtained by electronic integration of the flow signal derived from the pneumotachograph and a differential transducer (Siemens-Elema, Stockholm). The respiration of three rabbit pups in natural sleep was recorded by infant magnetometers (constructed by N. Peterson, Harvard University, MA), which were placed bilaterally with tape on the lower part of the thorax.

In three of the older anaesthetized animals, arterial blood pressure was monitored via a catheter inserted in one of the carotid arteries. Body temperature was maintained by an electric pad and by radiation from a heating lamp.

Drug administration. Intraperitoneal injections of drugs were made either through a teflon catheter placed intraperitoneally under urethane or ether anaesthesia or through a needle. Control injections of isotonic saline (0.1–0.3 ml) were made repeatedly, which did not affect the respiration.

In four pups, the fourth ventricle was exposed and substances, dissolved in mock CSF, were applied directly onto the exposed surface of the medulla as previously described (18). Application of mock CSF (pH 7.4) did not affect the respiration.

The adenosine analogue, L-PIA, was obtained from Boehrin-

Table 1. *Experimental series*

Part	Group	Route of administration of substances	Age (d) median (range)	*n*
I	Anaesthetized animals	IP	8 (3–23)	7
	Decerebrate animals	IP	5.5 (2–8)	5
II	Anaesthetized animals	Fourth ventricle	13 (4–14)	4
III	Animals in natural sleep	IP	2.5 (1–6)	3

ger, Mannheim. Theophylline was given as the ethylenediamine salt (aminophylline) from Hässle in Gothenburg.

Analysis of data. Because the pups had varying body weights, the results are expressed as an average percentage from controls with their respective standard deviations (% ± SD). Statistical analysis was done with the Willcoxon Rank sum test. *P* values less than 0.05 were considered as statistically significant.

RESULTS

Part I. In the first and main series of experiments rabbit pups between 2–23 d of age were studied under urethane anaesthesia ($n = 7$) or after decerebration ($n = 5$). The adenosine analogue L-PIA was administered intraperitoneally. The substance was given in increasing concentrations: 0.1, 0.5, 1.0, and 5.0 μmol/kg. The respiratory depression after L-PIA was essentially qualitatively the same in decerebrate and anaesthesized animals. The dose of 1 μmol/kg caused a marked depression in the youngest animals (Fig. 1) whereas a higher dose, *i.e.*, 5 μmol/kg led to irreversible apnea and death (Fig. 3). A 5-fold higher dose (5 μmol/kg) was usually required in the older animals (Fig. 2). The latter dose was thus, chosen for quantitative analyses of these experiments. The effect reached its maximum after about 20 min and persisted for at least 45 min, unless theophylline was given in which case the effect of L-PIA was completely reversed.

In Figure 3 (left panel) the effect of L-PIA in the whole series with intraperitoneal injection are summarized. After 20 min a marked decrease in the respiratory rate and minute ventilation was observed. Respiratory rate decreased 30.6 ± 13.6% and the minute ventilation decreased by 30 ± 9.2%. When then the antagonist theophylline (20 mg/kg) was administered, the respiration became normal in all animals except two pups, both of which were 3-d-old and suffered apnea and death.

Theophylline (20 mg/kg) was given first to five animals prepared in the same way before the administration of L-PIA (5 μmol/kg). Theophylline *per se* caused a small increase in respiratory rate and minute volume, but had no effect on tidal volume (Fig. 3). Administration of L-PIA after theophylline led to a significantly lower decrease in the minute volume of the theophylline-treated animals than it did in the theophylline + L-PIA treated animals (−25.8% *versus* −62.7%, $P < 0.01$ by Willcoxon test). Blood pressure and heart rate decreased after intraperitoneal administration of L-PIA in three older animals; blood pressure decreased on an average by 18% (range, 11–37%) after 15 min and heart rate by 28.6% (range, 10–54%).

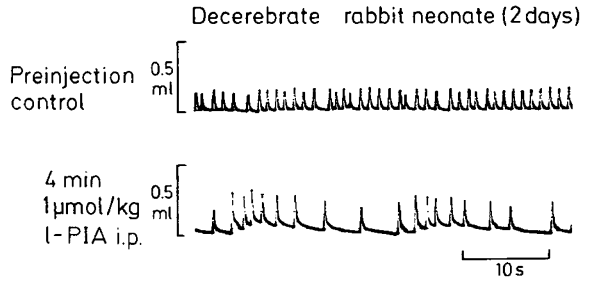

Fig. 1. The effect of L-phenyl-isopropyladenosine (L-PIA) (1 μmol/kg) on the respiration of a decerebrate rabbit neonate.

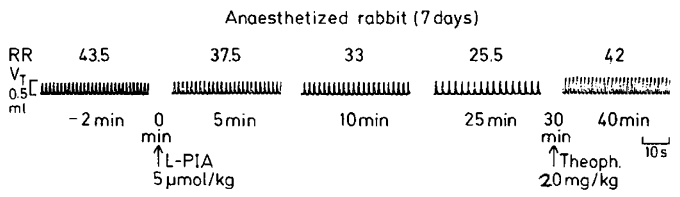

Fig. 2. The effect of L-phenyl-isopropyladenosine (L-PIA) and theophylline on tidal volume (V_T) and respiratory rate (RR).

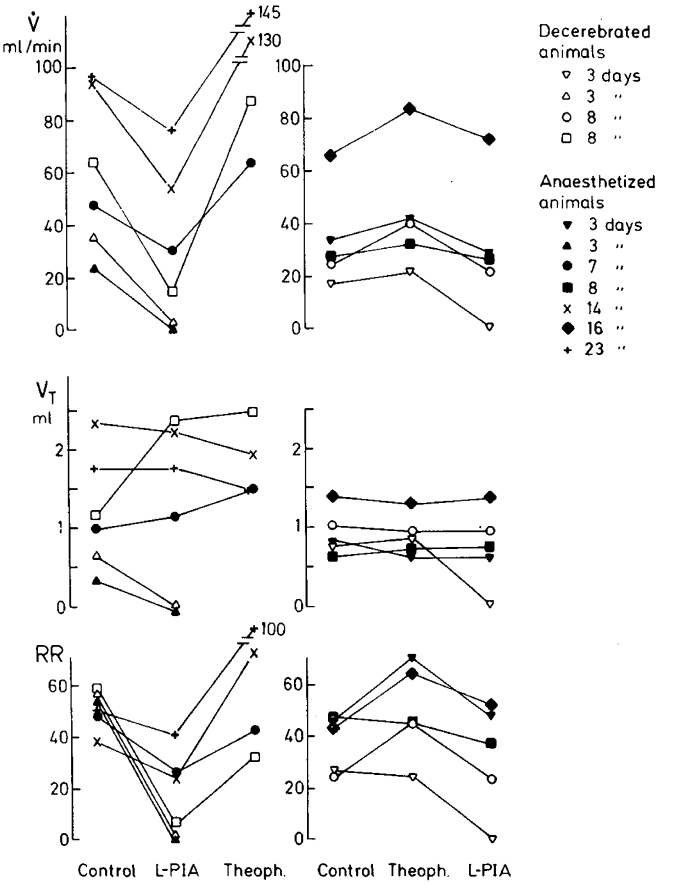

Fig. 3. The left panel shows the effect at 20 min after the administration of first L-phenyl-isopropyladenosine (L-PIA) (5 μmol/kg) and then 20 min later theophylline (20 mg/kg) on the minute volume ($\dot{V}$) tidal volume (V_T) and respiratory rate (RR). The right panel shows a second series of experiments when theophylline was first given and the L-PIA, with the same time intervals between the drugs. Three of the youngest animals died.

Part II. To investigate whether the respiratory depression of L-PIA may be caused by a direct effect on the bulbo-pontine control mechanisms or is secondary to systemic effects, L-PIA was administered directly onto the exposed surface of the fourth ventricle in four animals. A 10-fold lower dose than that used as systemic administration was sufficient to cause a comparable respiratory depression (Fig. 4). Reduction in minute ventilation (−41.8 ± 7.9%) was continuously observed in all four animals. This reduction seems to be due mainly to decreased respiratory rate (−31.8 ± 7.9%). The tidal volume decreased by −14.5 ± 4.4%.

Part III. The possibility that the anaesthetized and decerebrate animals were more susceptible to L-PIA than intact animals was also investigated. The respiration was monitored in three rabbit pups (age 1–6 d) during natural sleep using magnetometers. Even though the animals slept calmly enough to permit recording of respiration, quantitative measurements were difficult to perform. The depressive effect of L-PIA on respiration could be clearly demonstrated in all three animals. The effects were reversed by administration of theophylline (Fig. 5).

DISCUSSION

We studied the effects of adenosine agonists and antagonists on respiratory regulation of the rabbit neonate. Because adenosine is rapidly metabolized, a stable analogue, L-PIA, was used. This analogue is known to be a more selective agonist on central A1-adenosine receptors than other analogues, such as 2-chloroadenosine (9, 15).

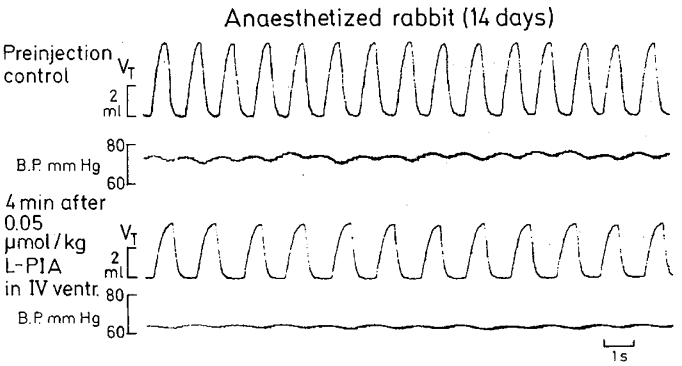

Fig. 4. The respiration and blood pressure before and after administration of L-phenyl-isopropyladenosine (L-PIA) into the exposed fourth ventricle (IV ventr.)

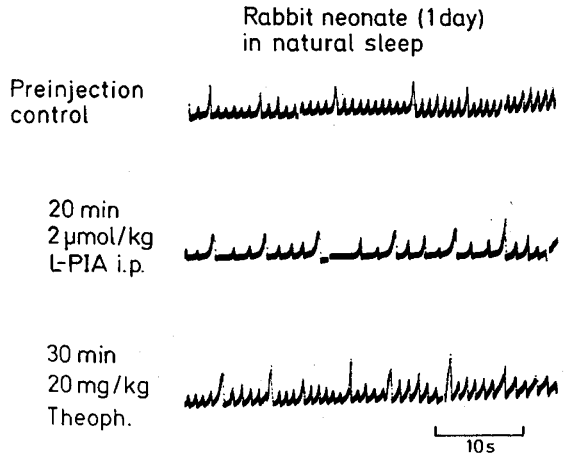

Fig. 5. The effect of L-phenyl-isopropyladenosine (L-PIA) and theophylline on the respiration of a 1-d-old rabbit neonate in natural sleep.

L-PIA caused a respiratory depression in all types of preparations of the rabbit pups. The respiratory frequency was particularly depressed, apparently due to a marked increase of expiratory duration. The effects on tidal volume were more variable, albeit the minute volume was always decreased.

The adenosine analogue could exert its effect on the respiratory control mechanisms by either directly affecting respiratory neurons in the brain stem or indirectly. Because the depression of ventilation induced by L-PIA was of the same order of magnitude and even tended to be more pronounced in the decerebrate animals than in the intact ones, the effects were probably not exerted at a suprapontine level. An effect via the peripheral chemoreceptors cannot be ruled out, but the strong effect by the direct application on the fourth ventricle shows that L-PIA has a direct action on the central respiratory neurons. Such an effect probably also occurs when L-PIA is administered systemically. Alternatively it is possible that hypotension in itself caused release of endogenous adenosine (17) leading to ventilatory depression. On the other hand hypotension would stimulate rather than inhibit respiration by removing tonic inhibitory influence from pressor receptors (7).

The youngest animals (2–3 d-old) tended to be more susceptible to the adenosine analogue than the older pups (10–14 d) after intraperitoneal administration. This could be due to the fact that the substance might reach the bulbo-pontine structures more easily in the young animals. The higher sensitivity to L-PIA in the youngest pups might also be due to a higher sensitivity of the central respiratory neurons because L-PIA passes the blood-brain barrier easily (4, 14). Furthermore, the younger animals tended to be more affected after administration of L-PIA in the fourth ventricle.

The antagonistic effect of theophylline was clearcut. Theophylline could prevent or even reverse the respiratory depression caused by L-PIA. Theophylline itself caused some stimulatory effect on respiration. This has been demonstrated in many studies in infants (1).

The present findings suggest that the therapeutic effect of theophylline could be exerted via blockade of adenosine receptors. This is further supported by some preliminary unpublished observations that the adenosine antagonist 8-phenyl-theophylline, which is essentially devoid of phosphodiesterase inhibitory effect (8, 14), was as potent as theophylline.

A corollary to that hypothesis is that adenosine may participate in the genesis of neonatal apnea. It is well known that hypoxia precipitates apnea in preterm infants (2, 12). It has been shown repeatedly that hypoxia increased the levels of adenosine in the brain (9, 11, 15, 17, 20). In the rat brain, the levels of extracellular adenosine reached during hypoxia were about 2–10 µM (20). These concentrations are sufficient to cause profound effects on central neurons (8, 9, 15). The levels of the major adenosine metabolic hypoxanthine have been shown to reflect the degree of asphyxia in newborn infants (13), suggesting also that under clinical conditions adenosine production may be sufficiently large to precipitate central actions. Recently, theophylline has been found to reduce ventilatory depression in hypoxic newborn piglets (3).

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Letter to the Editor

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We would like to comment on the interesting observation by P. Landrieu and coworkers (Pediatr. Res., *16:* 977, 1982). Contrary to the statement of the authors, "it seems unlikely that sodium valproate (SV) played an important role," we suggest that SV could have been of major importance in the development of acute hepatic failure for the following reasons. Liver function tests were stated to be normal before the start of SV. Three days after the start of SV blood ammonia and serum GPT rose and PTT decreased. It must be noted that SV was given in an unusually high dosage of 100 mg/(kg·d) [normal dosage, 10–20 mg/(kg· d)]. The changes in urinary amino acids between d 2 (start of SV) and d 3 cannot be explained by the OTC deficiency but are typical for SV therapy [increase of alanine and glycine, decrease of glutamic acid, glutamine and tyrosine (4, 5)]. Furthermore, there was an important decrease of orotic acid which can be explained by the inhibitory effect of SV on the first step of the urea cycle (2). By giving SV in high doses a second block was introduced into the urea cycle besides the existing OTC deficiency. The observed accumulation of fat droplets in the cytoplasm could be ascribed to SV which is known to cause steatosis (3) and to decrease serum levels of carnitine, necessary for the oxidation of fatty acids (1, 6). Recently, a boy with OTC deficiency died at the age of 13 mo after the introduction of SV [33 mg/(kg·day)] for febrile convulsions (8).

We agree with the authors that the changes in peroxisomes are unlikely to be specifically related to the OTC deficiency. The possibility that SV or SV in association with OTC deficiency caused the peroxisomal changes rather than the OTC deficiency itself cannot be excluded as SV is a short chain fatty acid and fatty acids are known to induce peroxisomal changes (7). It would be of interest to know whether the recovery of this patient occurred during SV therapy or after SV had been stopped or diminished.

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