Adenine Compounds: Cerebrovascular Effects
In Vitro with Reference to their Possible
Involvement in Migraine

J. E. HARDEBO, M.D. AND L. EDVINSSON, PH.D.

SUMMARY Adenosine and adenine compounds (AMP, cyclic AMP, ADP and ATP) markedly dilated feline and human pial arteries in vitro, the effect being more prominent with increasing tone of the vessel (active tonic contraction induced by prostaglandin F2α, or serotonin). In contrast, the various adenine compounds were unable to produce any dilatation of extracranial arteries tested (branches of lingual, external maxillary, and superficial temporal arteries). The degree of dilatation depended upon the perivascular potassium concentration, so that low potassium increased Emax and reduced ED50 values. Possible involvement of adenine compounds in the vasodilatory phase of the migraine attack is discussed.

THE VASCULAR REACTION during a migraine attack often comprises an initial constriction followed by a dilatation of large arteries. These circulatory changes have been particularly investigated in the extra- and intracranial circulation. Neurological deficits are not uncommon as a result of ischemia during the initial vasoconstrictory phase of the attack. This may cause metabolic changes, i.e. a perivascular increase of H+ and K+ as well as adenosine has been observed. Similarly, ischemia causes a rapid increase of adenosine and the related adenine compounds AMP and cyclic AMP in cerebral cortical tissue. The content of cyclic AMP in the cerebrospinal fluid, and hence its concentration in the vicinity of pial vessels, is enhanced during a migraine attack. Against this background it is of interest to determine whether adenosine and closely related adenine compounds (AMP, cyclic AMP, ADP and ATP) may cause a sufficiently high degree of vasodilatation in vitro to account for a possible involvement in initiating the vasodilatory phase of a migraine attack.

Methods
Twenty-four adult cats of either sex weighing 2 to 4 kg were used. The animals were killed by exsanguination under barbiturate (Nembutal, 30 mg/kg i.p.) anesthesia, the brain was removed and the middle cerebral arteries, as well as branches of extracranial arteries (lingual and external maxillary), were immediately dissected out. They were kept in aerated Krebs-Ringer (K-R) buffer solution of the following composition (millimolar concentrations): NaCl 118, KCl 4.5, MgSO4 × 2H2O 1.0, KH2PO4 1.0, NaHCO3 25, CaCl2 × 2H2O 2.5 and glucose 6.0. To increase the potassium concentration, K+ was substituted for Na+ to yield a concentration of KC1 of 9.0 mM. In a few experiments mock cerebrospinal fluid (CSF buffer) was used instead of K-R buffer solution; it had the following composition (mM): NaCl 120, KCl 3.0, MgCl2 0.29, NaH2PO4 0.50, Na2HPO4 0.25, NaHCO3 0.86 and glucose 6.0. Part of the material was immediately used in the experiments, whereas the rest was used after storage in the buffer solution at +4°C for up to 24 hours. Human vessels from 3 patients were obtained during neurosurgical operations. Segments of pial arteries were removed from normal parts of resected frontal and temporal lobes. In connection with the craniotomy, small branches were taken from the superficial temporal artery of the same patient. Pieces about 5 mm long from the dissected arteries were mounted between 2 L-shaped metal holders in a manteled organ bath for simultaneous recording of circular isometric tensions as described elsewhere. The tension was measured with force displacement transducers and recorded on a Grass polygraph. The bath contained either the K-R or the CSF buffer solution. It was maintained at 37°C (range 0.5°C) and continuously aerated with a mixture of 95% O2 and 5% CO2, giving a mean pH of 7.38 for the K-R buffer and 7.30 for the CSF buffer. The vessels were given a passive load of 400 dynes and allowed to attain a steady level of tension during a 2 hour accommodation period before testing.

In order to reveal a clear-cut dilatory response the vessels were given an active tonic constriction by either prostaglandin F2α (PGF2α; 2.5 × 10^-6M or 2.5 × 10^-5M) or 5-hydroxytryptamine (5-HT; 3 × 10^-6M), before the adenine compounds were administered to the organ bath by cumulative application.

Drugs
Drugs used in this study were: adenosine, AMP (adenosine 5'-monophosphate), cyclic AMP (adenosine 3'-5'-cyclic monophosphate), ADP (adenosine 5'-diphosphate), ATP (adenosine 5'-triphosphate), aminophylline and theophylline (all from Sigma), 2-2'-pyrridyl-isatogen tosylate (gift of...
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Dr. M. Spedding, School of Pharmacy, Sunderland, England), prostaglandin F2a (Astra), 5-hydroxytryptamine creatinine sulphate (Sigma).

Results

The effect of adenosine, AMP, cyclic AMP, ADP and ATP was first analyzed in the relaxed vessel, i.e. before it was subjected to an active tone. Under these conditions, all adenine compounds caused dilatation of the feline pial artery (table 1), whereas no effect was obtained in extracranial arteries. A more clear-cut dilatation of the preparation was revealed after the vessels had been given an active tone by PGF2a (see table). The PGF2a-induced contraction remained at a steady level of tension long enough to allow for cumulative application of the drugs to be tested. The amount of dilatation was proportional to the degree of active tone (PGF2a 2.5 × 10⁻⁵ M and 2.5 × 10⁻⁴ M resulted in a mean contraction of 112 and 320 dynes, respectively). The potency order comparing the E₅₀ values (concentration of agonist producing half maximum response), was ATP > AMP = ADP = adenosine > cyclic AMP (fig. 1). Dilatations were equally prominent when the active tone was produced by 5-HT (mean contraction 313 dynes) instead of PGF2a (table). Not even when the vessel had been given an active tone by PGF2a or 5-HT was it possible to reveal any substantial dilatory response by adenine compounds in the extracranial vessels (number of tests = 28). The results on experiments with human
Adenosine concentration (M)

ATP concentration (M)

FIGURE 2. Dilatations induced by adenosine in 3 different feline pial vessels given an active tone by PGF$_{2\alpha}$ $2.5 \times 10^{-6}$ M beforehand at various potassium concentrations. The maximum dilatation obtained at a K$^+$ concentration of 3.0 mM was set at 100 per cent. The mean $E_{50}$ values ($\pm$ SE) were for the 9.0 mM K$^+$ concentration (K-R buffer) $6.5 \pm 2.8 \times 10^{-1}$ (6 tests); 4.5 mM K$^+$ (K-R buffer) $4.7 \pm 1.5 \times 10^{-1}$ (7) and 3.0 mM K$^+$ (CSF buffer) $8.5 \pm 0.8 \times 10^{-1}$ (3). The corresponding $E_{50}$ values were for dilatations obtained with ATP $3.2 \pm 1.0 \times 10^{-1}$ (5), $1.9 \pm 0.6 \times 10^{-1}$ (10) and $6.0 \pm 1.3 \times 10^{-1}$ (4). $E_{\text{max}}$ values were reduced with increasing K$^+$ concentration.

arteries were principally the same as those obtained in feline arteries.

As shown in figure 2, an increase in the K$^+$ concentration from 4.5 mM to 9.0 mM resulted in a less pronounced dilatory effect of the adenine compounds (adenosine and ATP tested). On the other hand, the sensitivity of the vessels to adenine compounds was enhanced in a bath containing CSF buffer instead of K-R buffer (fig. 2), probably as a result of the change in K$^+$ concentration from 4.5 to 3.0 mM. It is our experience that the survival time of the vascular segments is shorter in the CSF buffer than in the K-R buffer; hence the majority of tests were performed in the K-R buffer.

The presence of theophylline and amphetamine (3 $\times$ 10$^{-4}$ - 3 $\times$ 10$^{-5}$M) in the organ bath caused a decreased vascular sensitivity particularly at the lower concentrations of the adenine compounds (adenosine and ATP tested). The outcome of the response may, in part, be related to the fact that theophylline and amphetamine, at the high concentrations, slightly reduced the amount of the PGF$_{2\alpha}$ induced active tone of the vessel. 2$^{2'}$-pyridyl-isatogen has been reported to antagonize the response to ATP on smooth muscle preparations. This substance (tested at a concentration from 3 $\times$ 10$^{-7}$ to 3 $\times$ 10$^{-6}$M) caused a slight, slowly developing relaxation of the pial vessel, but had no effect on the ATP-induced vascular dilatation in these concentrations.

Discussion

This study has shown that all adenine compounds tested cause a marked vasodilatation of feline and human pial — but not extracranial — arteries in vitro. Similarly, intracarotid infusion of ATP induces a marked increase in cerebral blood flow in the baboon, as does adenosine in the dog. Topical application of adenosine on pial arteries induces vasodilatation in the cat. The amount of dilatation is more marked when the tone is increased before testing. But even a markedly relaxed vessel (with a tangential tension in the wall estimated to be only about ¼ of the normal tension in vivo), dilates upon exposure to the adenine compounds. The dilatory capacity is not restricted to the large pial vessels presently tested but is, in relative terms, equally prominent in vessels with diameters ranging from about 50 to 300 $\mu$m. The vessels dilate to a similar degree irrespective of whether the foregoing constriction has been induced by 5-HT or PGF$_{2\alpha}$. This arrangement may mimic the events taking place during the vasoconstrictory phase of a migraine attack, in which a transient increase of circulating 5-HT and
prostaglandin is believed to occur. The dilatatory capacity is dependent on the potassium concentration; an increase in \( K^+ \) diminishes the dilatation. At a \( K^+ \) concentration of 3.0 mM (CSF buffer) the vessels became more sensitive upon exposure to adenyl compounds. The influence of a further decrease in \( K^+ \) on adenyl-mediated dilatations was not investigated, since only small diminutions of \( K^+ \) occur during physiological and pathophysiological conditions.

The influence of \( H^+ \) concentration on the vascular dilatory effect of adenosine has been investigated in pial arteries at local perivascular application. The effect of adenosine in an alkaline surrounding was not agreed with the findings obtained in this study. However, little is known about the cause of vasodilatation in migraine, and it may be different in these 2 vascular territories. A fall in circulating levels of vasoconstrictor agents such as 5-HT and noradrenaline has been demonstrated, and a local release of prostacyclin, causing vasodilatation, has been suggested. Ischemia during the vasoconstrictory phase will cause an increase of adenine compounds in the brain. It is generally considered that dilatation of extracranial vessels is one causative factor for the headache phase. The present results would indicate that adenine compounds do not contribute to the vasodilatation in this vascular territory. However, dilatation of intracranial vessels may cause headache. Since adenine compounds cause a marked dilatation of intracranial vessels, particularly in those with a high tone, it is possible that the compounds may initiate the dilatory phase of the attack or, in more general terms, reactive hyperemia in the intracranial circulation.

References

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Local Cerebral Blood Flow in the Conscious Rat As Measured with $^{14}$C-Antipyrine, $^{14}$C-Iodoantipyrine and $^{3}$H-Nicotine

K. OHNO, M.D., K. D. PETTIGREW, AND S. I. RAPOPORT, M.D.

SUMMARY Local cerebral blood flow (LCBF) in the conscious rat was estimated with one of 3 radio-tracers — $^{14}$C-antipyrine, $^{14}$C-iodeantipyrine or $^{3}$H-nicotine. A tracer was infused intravenously at a constant rate and blood concentration was followed until the animal was killed by decapitation. Tracer concentration in each of 14 brain regions. The Kety-Schmidt analysis was applied to the data for $^{14}$C-antipyrine and $^{14}$C-iodeantipyrine. The results confirmed findings of Sakurada et al. (1978) that $^{14}$C-iodeantipyrine provides LCBF's that are twice those obtained with $^{14}$C-antipyrine and that approximate LCBF's found with an inert gas. LCBF was calculated from the $^{3}$H-nicotine data by assuming complete extraction of tracer from blood by brain. This assumption was approximated for infusion times of 50 sec or less, when LCBF's were lower than 50-sec values due to back-diffusion, but nevertheless were as high as $^{14}$C-antipyrine LCBF's.

LOCAL CEREBRAL blood flow (LCBF) can be determined in experimental animals by employing an inert gas tracer, $^{131}$I-trifluoriodomethane, and applying the mathematical principles of Kety to analyze blood-brain exchange.5-8 The principles are valid because diffusional equilibrium between brain and blood is established almost instantaneously.5-8 Since use of a gas tracer presents technical difficulties, attempts have been made to employ non-gaseous tracers to measure LCBF. $^{14}$C-antipyrine has been used with some success, but it provides LCBF's which are less than those obtained using inert gases. Transfer of $^{14}$C-antipyrine from blood to brain is limited by the comparatively low diffusion coefficient of that tracer in the brain. The cerebrovascular PS (permeability X surface area) of $^{14}$C-antipyrine is about 0.020 cm$^3$ sec$^{-1}$ g$^{-1}$, and it is not sufficiently high to satisfy the assumptions of the Kety method.5-8 Early work suggested that $^{131}$I-iodeantipyrine might

From the Laboratory of Neurosciences, National Institute on Aging, Gerontology Research Center, Baltimore City Hospital, Baltimore, MD 21224 and Theoretical Statistics and Mathematics Branch, National Institute of Mental Health, Bethesda, MD 20014. Dr. Ohno is Visiting Fellow, Department of Neurosurgery, Tokyo Medical and Dental University, Tokyo, Japan. Reprints: Dr. Rapoport, Laboratory of Neurosciences, Gerontology Research Center, Baltimore City Hospital, Baltimore, MD 21224.
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J E Hardebo and L Edvinsson

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