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 E_max and reduced ED₆₀ 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.¹ ² 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.³ 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.⁴ 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 X 2H₂O 1.0, KH₂PO₄ 1.0, NaHCO₃ 25, CaCl₂ X 2H₂O 2.5 and glucose 6.0. To increase the potassium concentration, K⁺ was substituted for Na⁺ to yield a concentration of KCl 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 25, CaCl₂ 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.⁵ 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 × 10⁻⁷M) or 5-hydroxytryptamine (5-HT; 3 × 10⁻⁷M), 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
FIGURE 1. Representative log dose-response curves of dilatations induced by adenosine, cyclic AMP, ADP and ATP from consecutive tests on the same feline pial artery. The vessels had been given an active tone beforehand by PGF$_2\alpha$ 2.5 $\times$ 10$^{-5}$ M. The maximum dilatation induced by ATP was set at 100 percent.

Dr. M. Spedding, School of Pharmacy, Sunderland, England), prostaglandin F$_{2\alpha}$ (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 I), 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 PGF$_2\alpha$ (see table). The PGF$_{2\alpha}$-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 (PGF$_{2\alpha}$ 2.5 $\times$ 10$^{-5}$M and 2.5 $\times$ 10$^{-4}$M resulted in a mean contraction of 112 and 320 dynes, respectively). The potency order comparing the ED$_{50}$ 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 PGF$_{2\alpha}$ (table). Not even when the vessel had been given an active tone by PGF$_{2\alpha}$ 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) 10⁻⁹ 10⁻⁸ 10⁻⁷ 10⁻⁶ 10⁻⁵

ATP concentration (M)

FIGURE 2. Dilatations induced by adenosine in 3 different feline pial vessels given an active tone by PGF₂α 2.5 × 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 ED₅₀ values (± SE) were for the 9.0 mM K⁺ concentration (K-R buffer) 6.5 ± 2.8 × 10⁻⁴ (6 tests); 4.5 mM K⁺ (K-R buffer) 4.7 ± 1.5 × 10⁻⁴ (7) and 3.0 mM K⁺ (CSF buffer) 8.5 ± 0.8 × 10⁻⁴ (3). The corresponding ED₅₀ values were for dilatations obtained with ATP 3.2 ± 1.0 × 10⁻⁴ (5), 1.9 ± 0.6 × 10⁻⁴ (10) and 6.0 ± 1.3 × 10⁻⁴ (4). Eₘₐₓ values were reduced with increasing K⁺ concentration.

Students 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 aminophylline (3 × 10⁻⁷ - 3 × 10⁻⁴ 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 aminophylline, at the high concentrations, slightly reduced the amount of the PGF₂α induced active tone of the vessel. 2-2'-pyrridyl-isatogen has been reported to antagonize the response to ATP on smooth muscle preparations. This substance (tested at a concentration from 3 × 10⁻⁷ to 3 × 10⁻⁴ 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 1% 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 30 to 300 µ. The vessels dilate to a similar degree irrespective of whether the foregoing constriction has been induced by 5-HT or PGF₂α. 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.\textsuperscript{14, 15} The dilatary capacity is dependent on the potassium concentration; an increase in K\textsuperscript{+} diminishes the dilatation.\textsuperscript{16} At a K\textsuperscript{+} 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\textsuperscript{+} on adenylylated dilatations was not investigated, since only small diminutions of K\textsuperscript{+} occur during physiological and pathophysiological conditions.\textsuperscript{17, 18}

The influence of H\textsuperscript{+} concentration on the vascular dilatory effect of adenosine has been investigated in pial arteries at local perivascular application.\textsuperscript{19} The effect of adenosine in an alkaline surrounding was not diminished during alkalosis.\textsuperscript{19} The order of potency of adenine compounds in causing relaxation has been evaluated in coronary vessels. Most workers find ATP and ADP to be the most potent agents on the coronary vasculature; AMP, adenosine and cyclic AMP are one-fourth to one-third less potent than ATP.\textsuperscript{20} This is principally in agreement with the findings obtained in this study. It is notable that ATP administered systemically has effects beyond the blood-brain barrier: the observed increase in cerebral blood flow is accompanied by an increase in CMRO\textsubscript{2}.\textsuperscript{9} In this context it should be recalled that ADP may be released from platelets during the early phase of a migraine attack in conjunction with an increased platelet aggregability.\textsuperscript{21, 22}

Administration of the phosphodiesterase inhibitor, aminophylline, has been suggested as a possible way to treat cerebral vasospasm.\textsuperscript{23} The vasodilatory effect resulting from the increase in adenine compounds may, however, be partly counteracted by the presently observed inhibitory effect of aminophylline on the adenine-mediated dilatations.\textsuperscript{11, 24}

There is at present no convincing evidence for an interaction of adenine compounds with specific adenosine receptors. A neural localization of ATP in purinergic nerves has been suggested by Burnstock.\textsuperscript{20} It is possible that adenine compounds exert their vasodilatory effect by a direct action on the smooth muscle cells in the vessel wall.\textsuperscript{20} Whatever this mechanism is, a marked difference exists between the intra- and extracranial carotid vasculature, in the sense that no clearcut dilatation was obtained in the extracranial arteries under the present in vivo conditions. Further, the action is apparently dependent upon the perivascular concentrations of K\textsuperscript{+} and H\textsuperscript{+} and on the present state of vascular tone. It is generally accepted that both intracranial and extracranial vessels dilate following the constrictor phase of a migraine attack.\textsuperscript{1, 2} 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\textsuperscript{14} and noradrenaline\textsuperscript{28} has been demonstrated, and a local release of prostacyclin, causing vasodilatation, has been suggested.\textsuperscript{27} 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.\textsuperscript{28} Since adenine compounds cause a marked dilatation of intracranial vessels, particularly in the territory with a high tone, it is possible that the compounds may initiate the dilatation phase of the attack or, in more general terms, reactive hyperemia in the intracranial circulation.

References

16. Wahl M, Kuschinsky W: Influence of H\textsuperscript{+} and K\textsuperscript{+} on adeno-
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-iendoantipyrine 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 was then measured in each of 14 brain regions. The Kety-Schmidt analysis was applied to the data with $^{14}$C-antipyrine and $^{14}$C-iendoantipyrine. The results confirmed findings of Sakurada et al. (1978) that $^{14}$C-iendoantipyrine 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 derived with $^3$H-nicotine generally did not differ significantly from LCBF's obtained with $^{14}$C-iendoantipyrine. Fifty-sec $^3$H-nicotine-derived flows for the pineal and pituitary glands were, respectively, $2.59 \pm 0.36$ (SEM) cm$^3$ g$^{-1}$ min$^{-1}$ and $1.28 \pm 0.08$ cm$^3$ g$^{-1}$ min$^{-1}$. LCBF's calculated for 70 sec to 240 sec of $^3$H-nicotine infusion were lower than 50-sec values due to back-diffusion, but nevertheless were as high as $^{14}$C-antipyrine LCBF's due to the marked binding of tracer by brain tissue. The results supported the conclusion of Sakurada et al. (1978) that iendoantipyrine is the non-gaseous agent of choice for measuring LCBF precisely, but also showed that short infusion schedules with nicotine provided good estimates of LCBF.

Stroke, Vol 10, No 1, 1979
Adenine compounds: cerebrovascular effects in vitro with reference to their possible involvement in migraine.
J E Hardebo and L Edvinsson

doi: 10.1161/01.STR.10.1.58

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1979 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/10/1/58

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Stroke* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Stroke* is online at:
http://stroke.ahajournals.org//subscriptions/