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

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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 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. 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 X 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 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. 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 × 10⁻⁶M), 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'-pyridyl-isotogen tosylate (gift of
Adenine compound concentration (M)

**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$_{2a}$ $2.5 \times 10^{-4}$ M. The maximum dilatation induced by ATP was set at 100 percent.

Dr. M. Spedding, School of Pharmacy, Sunderland, England), prostaglandin F$_{2a}$ (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 PGF$_{2a}$ (see table). The PGF$_{2a}$-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$_{2a}$ $2.5 \times 10^{-4}$M and $2.5 \times 10^{-5}$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$_{2a}$ (table). Not even when the vessel had been given an active tone by PGF$_{2a}$ 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...
arteries were principally the same as those obtained in
feline arteries.

As shown in figure 2, an increase in the K+ concen-
tration 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 ex-
perience 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
\((3 \times 10^{-9} - 3 \times 10^{-4} M)\) in the organ bath caused
a decreased vascular sensitivity particularly at the lower
concentrations of the adenine compounds (adenosine
and ATP tested, fig. 3). 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 PGF\(_{2\alpha}\) 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 concentra-
tion 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 \(\frac{1}{3}\) 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 \(\mu\). 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.\textsuperscript{14, 16}

The dilatatory capacity is dependent on the potassium concentration; an increase in $K^+$ diminishes the dilatation.\textsuperscript{16} 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 adenyln-mediated dilatations was not investigated, since only small diminutions of $K^+$ occur during physiological and pathophysiological conditions.\textsuperscript{17, 18}

The influence of $H^+$ concentration on the vascular dilatory effect of adenosine has been investigated in pial arteries at local perivascular application.\textsuperscript{18} The effect of adenosine in an alkaline surrounding was not altered, but it was decreased in an acidic milieu. In the coronary circulation, however, the dilatory effect of adenosine was enhanced during systemic acidosis and diminished during alkalosis.\textsuperscript{18}

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$_2$.\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}


Somerville BW: Platelet-bound and free serotonin levels in jugular and forearm venous blood during migraine. Neurology (Minneapolis) 26: 41–45, 1976


Wahl M, Kuschinsky W: Influence of H$^+$ and K$^+$ on adeno-
Local Cerebral Blood Flow in the Conscious Rat As Measured with $^{14}$C-Antipyrine, $^{14}$C-Iodoantipyrine and $^{3}$H-Nicotine

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SUMMARY Local cerebral blood flow (LCBF) in the conscious rat was estimated with one of 3 radioactive tracers — $^{14}$C-antipyrine, $^{14}$C-iodoantipyrine 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 brain was then measured in each of 14 brain regions. The Kety-Schmidt analysis was applied to the data with $^{3}$H-antipyrine and $^{14}$C-iodoantipyrine. The results confirmed findings of Sakurada et al. (1978) that $^{14}$C-iodoantipyrine provides LCBF's that are twice those obtained with $^{3}$H-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.

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. $^{1-4}$ The principles are valid because diffusional equilibrium between brain and blood is established almost instantaneously. $^{5,6}$ 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.

Early work suggested that $^{131}$I-iodoantipyrine might...
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