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Demonstration of Adenosine Receptors on Mouse Cerebral Smooth Muscle Membranes
DAVID W. BECK, M.D., HARRY V. VINTERS, M.D., STEVEN A. MOORE, M.D., PH.D.,
MICHAEL N. HART, M.D., FRITZ A. HENN, M.D., PH.D., PASQUALE A. CANCELLA, M.D.

SUMMARY Adenosine receptors have been identified on brain cortical membranes and microvascular preparations. However, they have not been demonstrated on specific microvascular elements in isolation. 2-
H-chloroadenosine was used as a ligand to investigate the presence of adenosine receptors on isolated mouse cerebral smooth muscle membranes. The binding studies reveal the presence of a high affinity binding site with a Kd value of 33.3 nM and a maximal binding capacity (Bmax) of 283 fmol/mg protein. These findings demonstrate that there is an adenosine receptor on cerebral smooth muscle membranes.

ADENOSINE has been proposed as a neuroregulator of cerebral blood flow1-2 because of the rapid rise in brain adenosine levels following hypotension, ischemia, seizures, and hypoxia4 and because adenosine is a potent vasodilator.5 In its proposed role of regulating cerebral blood flow it is speculated that adenosine may be produced at the glial foot process via 5' nucleotidase,6 released into the extracellular space, and bound to cerebral smooth muscle membranes resulting in relaxation of the vessel.7 There is some evidence to support this hypothesis. Adenosine receptors A1 and A2 have been identified on brain membranes8 10 12 and are associated respectively with a decrease and an increase in cyclic AMP production.11 Furthermore, increased smooth muscle cyclic AMP has been associated with vessel relaxation.12 Adenosine receptors on cerebral smooth muscle membranes, however, have never been demonstrated. Palmer, et al13 have shown adenosine receptors in capillaries of rat cerebral cortex coupled to adenylate cyclase. However, these microvessel preparations are impure, in that they contain several microvascular elements such as glia, endothelium, smooth muscle, and pericytes.

If indeed adenosine regulates cerebral blood flow via action on cerebral smooth muscle membranes, then receptors on specific microvascular elements need to be demonstrated in isolation. We report the results of binding studies of 2-
H-chloroadenosine with isolated mouse cerebral smooth muscle membranes. Our results show a high affinity receptor for 2-
H-chloroadenosine is present on cerebral smooth muscle membranes.

Methods
Cell Cultures
Isolation of mouse cerebral microvessels and derivation of cerebral smooth muscle cells and cerebral endothelial cells in tissue culture have previously been described from our laboratory.14-16 Cerebral smooth muscle cells are characterized by their broad, polygonally shaped morphology and possess many characteris-
tics of smooth muscle: basal laminae, clusters of pinocyto
tic vesicles, and bundles of thin filaments. Isoelec
tropic focusing and SDS electrophoresis of cellular
proteins on polycrylamide gels after label-
ing the cultures with [S-35]-methionine demonstrates
that these cells actively synthesize g-actin, a smooth
muscle specific isoactin. No cells in our cultures
stained with either anti-factor VIII antibody, or anti-
GFAP, an astrocyte specific antibody. It is therefore doubtful that our cul-
tures were contaminated with other brain cell types.
Stock cultures of the cell lines were maintained in
modified Lewis media (MLM17 supplemented with
20% fetal bovine serum and were passed twice weekly.
All studies were performed on cells prior to passage
10.

Preparation of membranes
Cells were grown to confluence in 100 mm tissue
culture dishes, scraped from the plastic with a "rubber
policeman", and placed into centrifuge tubes with T
Tris-HCl and resuspended. Microvessel fractions using 2-chloroadenosine as a li-
gand. However, these preparations are impure, includ-
ing elements such as pericytes, endothelium, smooth
muscle, and glia. We have demonstrated a high affinity
adenosine receptor for 2H-chloroadenosine on cere-
bral smooth muscle membranes with a Kd (33.3 nM)
and Bmax (283 fmol/mg protein). This binding is in
close agreement with that reported by Wu and Phil-
lish19,20 for 2H-chloroadenosine binding onto rat cere-
bral cortical synaptosomal membranes (Kd = 23.5
nM). Williams and Risley21,22 reported that 2H-
choloroadenosine bound to deaminase treated rat brain
membranes at two high affinity sites with Kd values of
1.31 nM and 16.2 nM. Our data is in agreement with
the lower of the two (16.2 nM) affinities. The binding
of 2H-chloroadenosine to microvessel fractions (Kd
= 0.038 nM) is a higher affinity binding and may
represent the first binding site (Kd = 1.31 nM) report-
ed by Williams and Risley. There are at least two
extracellular adenosine receptors associated with ade-
nylate cyclase activity, and it is unclear whether 2H-
choloroadenosine binds to the adenosine A1-receptor,
associated with a decrease in cyclic AMP accumula-
tion, or the adenosine A2-receptor, associated with an
increase in cyclic AMP accumulation.19,20

Adenosine, a potent vasodilator, has been proposed as a
neuroregulator of cerebral blood flow via action on
cerebral smooth muscle membranes.1-2 Our findings of
a high affinity adenosine receptor on isolated cerebral

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**Figure 1a. Bindings of 2H-chloroadeno-
sine to mouse cerebral smooth muscle
membrane. The points are the mean of triplicate
determinations of three separate experiments
with SD indicated by vertical bars. b. Scat-
chard plot of (a). The line was generated by lin-
ear regression (r = 0.96) yielding values for
Kd = 33.3 nM and Bmax = 283 fmol/mg protein.**

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smooth muscle membranes are consistent with this hypothesis. The action of adenosine on the brain microvasculature is undoubtedly complex and its interaction with specific microvascular elements is unclear. It has been shown that adenosine is taken up by isolated microvessels, cerebral endothelium and cerebral smooth muscle via a carrier-mediated system. Also, the affinity of uptake into isolated cerebral endothelium (Km = 5.0 μM) is greater than that into isolated cerebral smooth muscle cells (Km = 10.0 μM). These data suggest that cerebral endothelium may serve to regulate extracellular adenosine concentrations while cerebral smooth muscle serves a more complex role: adenosine may bind to its membrane receptor, possibly resulting in increased intracellular cyclic AMP and vessel relaxation.

Conclusion

We have demonstrated a high affinity adenosine receptor on cerebral smooth muscle membranes. These results are consistent with the hypothesis that adenosine regulates cerebral blood flow via action on cerebral smooth muscle.

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