β-Adrenergic–Sensitive Adenylate Cyclase in Secretory Cells of Choroid Plexus

Abstract. *Biochemical evidence supporting the sympathetic control of cerebrospinal fluid production has been obtained through identification of a specific β-adrenergic-sensitive adenylate cyclase in the choroid plexus. The enzyme, which is localized in the secretory epithelium, is activated by low concentrations of isoproterenol and norepinephrine and appears separate from β-adrenergic-sensitive adenylate cyclase present in cerebral blood vessels.*

The choroid plexus plays an important role in cerebrospinal fluid (CSF) production, but, as yet, control mechanisms for CSF secretion by the choroid are poorly understood (*1, 2*). Movement of water, electrolytes, and other substances from blood to cerebral ventricles occurs across the choroid through a network of capillaries covered by a secretory epithelium (*2*). Histological studies demonstrate that both capillaries and epithelium receive noradrenergic innervation from the sympathetic chain (*3, 4*), stimulation of which has been shown to alter CSF pressure and production (*4, 5*). In certain other tissues (such as salivary gland and ciliary body) known to receive sympathetic innervation, adrenergic effects (on secretion) appear to be mediated through a β-adrenergic receptor closely coupled to a membrane-bound adenylate cyclase (*6*). Binding of β-adrenergic agonists to such receptors leads to the intracellular synthesis of adenosine 3′,5′-monophosphate (cyclic AMP) (*7*). Recent studies of CSF secretion in the dog (*8*) have shown that intraventricular cholera toxin, a known activator of adenylate cyclase, markedly increases CSF production. Taken together, these facts suggest that noradrenergic mechanisms could play a role in the regulation of CSF production. If so, it would be of considerable interest to determine whether a β-adrenergic receptor coupled to adenylate cyclase is present in the choroid plexus and whether it is associated not only with vascular components but with the secretory epithelium itself (*9*). Identification of such an enzyme would supply evidence for a functional role of β-adrenergic receptors in the choroid and would facilitate the evaluation of the actions of pharmacological agents on CSF secretion.

To test this supposition, the effects of various biogenic amines were studied on the enzymatic synthesis of cyclic AMP from adenosine triphosphate (ATP) in broken cell preparations of cat, rabbit, calf, and dog choroid plexus. Figure 1 shows that *l*-isoproterenol (ISO), a β-adrenergic agonist, caused marked stimulation of adenylate cyclase activity (as much as 370 percent of control) in a homogenate of cat choroid plexus. Concentrations of ISO as low as $10^{-8}M$ caused significant stimulation, and half-maximal activation occurred at a concentration (K_a) of $2 \times 10^{-7}M$. *l*-Norepinephrine (NE), a mixed β- and α-adrenergic agonist, stimulated enzyme activity nearly as much as ISO (310 percent), but was less potent, with a K_a of $3 \times 10^{-5}M$. The α-adrenergic agonist, *l*-phenylephrine, was both less potent and less effective, with a K_a of more than $10^{-4}M$ and a maximal activation of 185 percent of control levels. The relative potency of these three amines was the same in several other experiments, although maximal enzyme stimulation (in the cat) varied between 270 and 490 percent of control. In the rabbit, maximal stimulation by ISO averaged 260 percent (two experiments); in the cow, 220 percent (four experiments); and in the dog, 210 percent (one experiment).

Table 1. (a) Effects of the β-adrenergic antagonist propranolol, the α-adrenergic antagonist phentolamine, and the dopamine antagonist fluphenazine on stimulation of cat choroid plexus adenylate cyclase activity by isoproterenol. (b) Effects of maximally effective concentrations of various amines, alone and in combination, on choroid plexus adenylate cyclase activity in a preparation from another cat perfused systemically prior to choroid removal. (Qualitatively similar data were obtained with tenfold lower amine concentrations.) The values shown in (a) are the increases due to ISO over enzyme activity in the presence of antagonist alone. In the absence of added agents, control activity was 13.0 ± 0.6 pmole per milligram of protein per minute in (a) and 14.9 ± 0.9 pmole per milligram of protein per minute in (b).

Agent	Cyclic AMP increase (pmole/mg-min)
(a) Antagonists	
Isoproterenol (100 μ*M*)	21.1 ± 1.5
Isoproterenol (100 μ*M*) plus propranolol (50 μ*M*)	2.4 ± 1.7
Isoproterenol (100 μ*M*) plus phentolamine (50 μ*M*)	20.9 ± 0.8
Isoproterenol (100 μ*M*) plus fluphenazine (50 μ*M*)	20.7 ± 1.5
(b) Additivity	
Isoproterenol (1 m*M*)	25.5 ± 1.0
Norepinephrine (1 m*M*)	20.0 ± 2.4
Dopamine (1 m*M*)	7.5 ± 0.5
Histamine (1 m*M*)	27.4 ± 0.7
Isoproterenol (1 m*M*) plus norepinephrine (1 m*M*)	22.4 ± 0.3
Isoproterenol (1 m*M*) plus dopamine (1 m*M*)	25.3 ± 0.1
Isoproterenol (1 m*M*) plus histamine (1 m*M*)	42.9 ± 0.3

Results similar to those in Fig. 1 were obtained with choroids from cats which had been first perfused systemically to remove intravascular blood (Table 1b). Since, in addition, whole blood itself showed little ISO-stimulated enzyme activity, it seems unlikely that blood elements were contributing to the results. In contrast to its large stimulatory effects on choroid adenylate cyclase, ISO caused only a small (128 percent of control) increase of enzyme activity in homogenates of whole cat brain.

The preferential activation of a β-adrenergic receptor by ISO in the choroid was supported by other experiments with receptor antagonists. Table 1 shows that ISO-stimulated adenylate cyclase activity was completely blocked by the β-adrenergic antagonist propranolol, but was unaffected by similar concentrations of the α-adrenergic antagonist phentolamine or the potent dopamine antagonist fluphenazine. Histamine, known to stimulate adenylate cyclase in brain (*10*), also activated choroid adenylate cyclase (200 to 300 percent of control; K_a of 12 μ*M*). Histamine stimulation was partially additive to that caused by ISO (Table 1) and was not blocked by propranolol (data not shown), suggesting that histamine and ISO were affecting distinct receptors. In contrast, NE stimulation was not additive to that caused by ISO (Table 1) but was blocked by propranolol, suggesting that the effect of NE on choroid adenylate cyclase was through its action on β-adrenergic receptors. Dopamine also increased enzyme activity to a small degree, but no additive effects were seen between this amine and ISO (Table 1).

Because the choroid plexus is highly vascularized, it was of interest to examine the effects of ISO on adenylate cyclase activity in other cerebral vasculature. In homogenates of cat extraparenchymal pial vessels and in homogenates of purified intraparenchymal microvessels (*11*), ISO-sensitive adenylate cyclase was present (*12*), but basal enzyme activity as well as the degree of ISO stimulation were about 25 percent of those in cat choroid plexus. This finding, along with the observation that the effects of guanosine 5′-triphosphate (GTP) on adenylate cyclase were qualitatively different in the cho-

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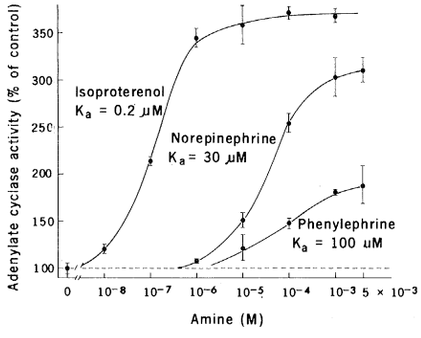
roid and in nonchoroidal vasculature, suggested that choroid vascular elements might not be the major contributor to the ISO stimulation seen. This was supported further by the finding that the cat fourth ventricular choroid plexus, which contains a great deal of secretory epithelium, showed a twofold greater response to ISO (up to 800 percent of control) than the lateral ventricular choroid, which is more vascular.

To further localize β-adrenergic-sensitive adenylate cyclase, several procedures were employed to separate the superficial secretory epithelium of the choroid from the underlying capillary network. In Fig. 2a (see legend for details), cat choroid was separated into an epithelial cell and a capillary fraction by mechanical fragmentation and discontinuous sucrose gradient centrifugation, using a method similar to that employed for isolating cerebral microvessels from brain (*11, 12*). In Fig. 2b (cat choroid), the epithelial cells were separated from the capillary network by partial enzymatic digestion of the extracellular matrix; and in Fig. 2c (rabbit choroid), epithelial cells were dissociated from the underlying vessels by incubating in a Ca^{2+}-free, EDTA Ringer solution. In each case, basal and ISO-stimulated enzyme activities were greater in cell than vessel fractions, findings which support the presence of β-adrenergic-sensitive adenylate cyclase in choroid plexus secretory epithelium.



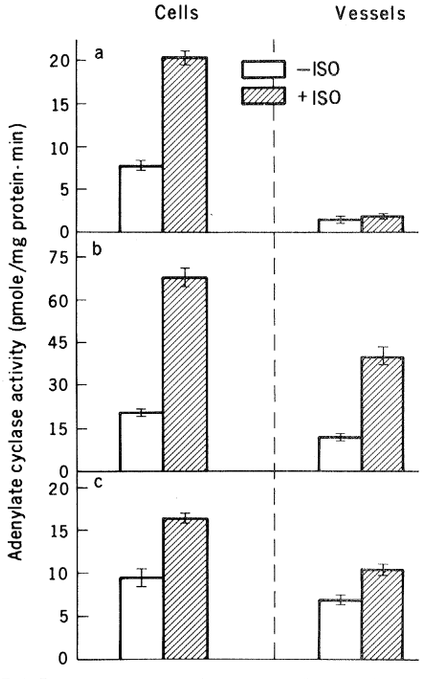


Fig. 1 (left). Effects of *l*-isoproterenol, *l*-norepinephrine, and *l*-phenylephrine on adenylate cyclase activity in a homogenate of lateral and fourth ventricle cat choroid plexus (control activity = 16.1 ± 0.4 pmole per milligram of protein per minute). The values shown here and in Fig. 2 and Table 1 are the means (± mean deviations) of replicate samples, each assayed for cyclic AMP in triplicate. For these studies, choroid plexus was dissected from animals which had been killed by air embolization or exsanguination following ether or nembutal anesthesia, rinsed in phosphate-buffered saline (PBS), and homogenized (15 mg/ml) in cold 6 m*M* tris maleate, *p*H 7.4. In some cases, animals were first perfused transcardially with 1000 ml of cold PBS. Cyclic AMP formation was measured in tubes containing (in 0.3 ml) 80 m*M* tris maleate, *p*H 7.4, 10 m*M* theophylline, 6 m*M* $MgSO_4$, 0.1 m*M* GTP, 1.5 m*M* ATP, and tissue homogenate (1 mg, wet weight), plus test substances as indicated. The reaction (4 minutes at 30°C) was initiated by addition of ATP and stopped by heating to 90°C for 2 minutes, followed by centrifugation to remove insoluble matter. Cyclic AMP was measured as in (*14*) and protein determined as in (*15*). Under these conditions, enzyme activity was linear with time and enzyme concentration, and phosphodiesterase activity was nearly completely inhibited. In some experiments, incubations were done in the presence of 0.1 percent ascorbic acid to reduce the possibility of differential oxidation of the various amines. Results were similar to those above. Fig. 2 (right). Localization of basal and isoproterenol-stimulated adenylate cyclase activity in epithelial cell and vascular fractions obtained from intact choroid plexus by three methods. (a) Cat choroid was gently fragmented by hand in a Teflon-glass homogenizer (0.2 mm clearance) in 2 ml of cold 25 m*M* tris maleate buffer, *p*H 7.4, containing 140 m*M* NaCl and 0.5 percent bovine serum albumin (BSA), and then centrifuged (25,000 rev/min for 35 minutes) on a discontinuous 1.0, 1.35, 1.5, 1.7, and 2.25*M* sucrose gradient. The vessel fraction (1.7 to 2.25*M* interface) was washed and collected against a 100-μm Nitex screen, while the remaining, less dense, nonvessel fractions were combined, diluted with medium, and collected by centrifugation at 50,000*g*. Both fractions were homogenized as in Fig. 1. (b) Cells were obtained by incubating cat choroid in 10 m*M* Hepes buffer, *p*H 7.4, containing 137 m*M* NaCl, 2.7 m*M* KCl, 0.7 m*M* Na_2HPO_4, 5.6 m*M* glucose, 1 percent BSA, 0.05 percent hyaluronidase, and 0.012 percent collagenase (three changes, 15 minutes each, at 37°C), after which 10 percent horse serum was added. The cells in suspension were washed twice, filtered through 100-μm Nitex, pelleted, and homogenized as in Fig. 1. The remaining, partially de-epithelialized capillary network was washed and homogenized as above. (c) Cells were dissociated from underlying capillaries by incubating rabbit choroid in 10 m*M* Hepes buffer, *p*H 7.4, containing 137 m*M* NaCl, 2.7 m*M* KCl, 0.7 m*M* Na_2HPO_4, 5.6 m*M* glucose, 1 percent BSA, and 2.25 m*M* EDTA (three changes, 20 minutes each, at 4°C), with intermittent trituration. Dissociated cells and remaining partially de-epithelialized choroid were washed and collected as in (b). Parallel histological studies indicated that the procedure in (a) gave the most complete dissociation of cells from vessels, whereas that in (c) caused the least disruption of the capillary network but left the greatest amount of epithelium remaining on the vessels. Results similar to those above were obtained in calf choroid plexus separated by these same methods.

These studies, in broken cell preparations of both whole and dissociated choroid, indicate the existence of β-adrenergic receptors closely associated with adenylate cyclase. Although a substantial portion of this enzyme was present in epithelial cell fractions, an influence of adrenergic mechanisms on choroid vasculature should not be excluded. It is known that catecholamines can affect the caliber of choroidal vessels (*5, 13*), and an adenylate cyclase stimulated by NE has been identified in cerebral vasculature (*12*). Additional studies of the physiological and biochemical consequences of activation of β-adrenergic-sensitive adenylate cyclase in the choroid should help to clarify the relative roles of secretory and vascular components in the sympathetic control of CSF production. Meanwhile, further characterization of the choroid β receptor itself may prove helpful in evaluating the potential effects of adrenergic agents on CSF production.

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References and Notes

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