Cerebroventricular Propranolol Elevates Cerebrospinal Fluid Norepinephrine and Lowers Blood Pressure

Abstract. *Ventriculocisternal administration of* dl- *and* d-*propranolol produced dose-dependent increases in cerebrospinal fluid norepinephrine and reductions in blood pressure. A highly significant correlation was found between the increase in norepinephrine and the hypotensive effect. The propranolol-induced hypotension was prevented by intracisternal phentolamine. These data indicate that the hypotensive effect of centrally administered propranolol results from a drug-induced release of norepinephrine, which stimulates central alpha receptors to lower arterial pressure.*

The role of central noradrenergic mechanisms in the regulation of blood pressure is well documented. Histochemical mapping demonstrates a close anatomical association between central noradrenergic neurons and brain areas that regulate cardiovascular function. Axons from noradrenergic cell bodies ascend into the hypothalamus, while descending tracts project into the spinal cord to synapse with sympathetic preganglionic neurons. A high density of noradrenergic nerve terminals is found in regions of the brainstem involved in cardiovascular control. Moreover, administration of norepinephrine (NE) into the central nervous system decreases blood pressure and heart rate. These centrally mediated hemodynamic effects of NE can be prevented by alpha-adrenergic antagonists, suggesting the involvement of central alpha-adrenergic receptors in cardiovascular control mechanisms (*1*, *2*).

The results of studies on the antihypertensive drugs clonidine and methyldopa are also consistent with the hypothesis that central catecholamine-containing neurons participate in the regulation of arterial blood pressure. Clonidine, for example, reduces peripheral sympathetic activity and lowers arterial blood pressure through stimulation of alpha-adrenergic receptors in the medullary area of the brain (*2*, *3*). The antihypertensive effect of methyldopa also appears to be the result of central alpha-adrenergic stimulation mediated by its metabolite methylnorepinephrine (*4*, *5*). Thus, studies with the natural neurotransmitter NE as well as with certain antihypertensive drugs indicate that activation of alpha receptor mechanisms in the brainstem results in reductions in peripheral sympathetic tone and arterial blood pressure.

An action within the central nervous system has been postulated to explain the antihypertensive effects of propranolol. Administration of propranolol into the cerebroventricular system in a number of animal species produces a prolonged reduction in systemic arterial pressure at doses that are ineffective when injected into the peripheral circulation (*6*, *7*). This effect results from a centrally mediated reduction in peripheral sympathetic activity (*7*, *8*). However, the mechanism underlying the central action of propranolol is not clear; neither its beta receptor blocking properties nor its local anesthetic action appears to be the basis of the hypotensive effect (*4*, *6*, *7*). Alternatively, there is evidence that this effect of propranolol might result from a presynaptic interaction of the drug at noradrenergic nerve terminals. Prior treatment with reserpine or 6-hydroxydopamine prevents the centrally mediated hypotensive effect (*6*, *9*). Moreover, propranolol can induce the release of NE from adrenergic nerve endings in the dog heart when the drug is injected directly into the coronary circu-

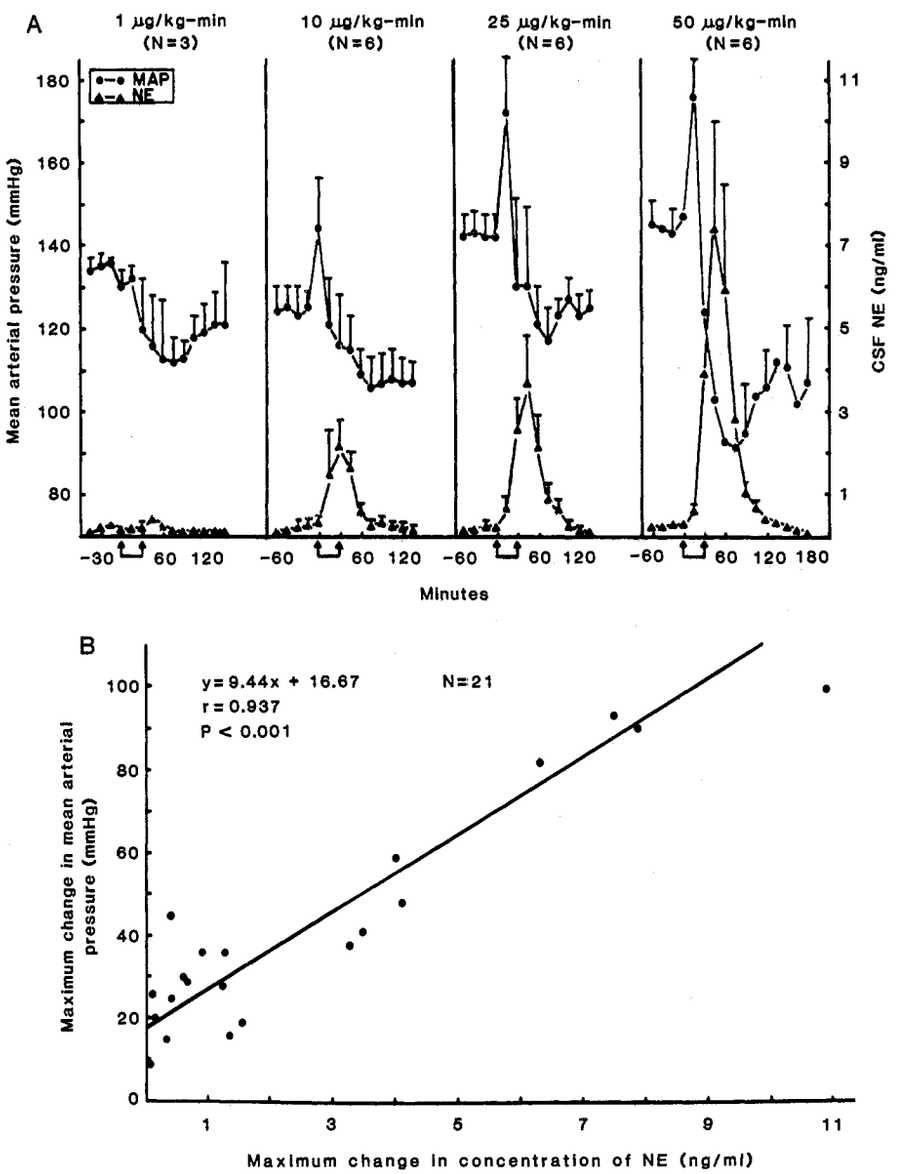

Fig. 1. (A) Effects of ventriculocisternal perfusion of *dl*-propranolol on mean arterial pressure (●) and CSF NE (▲). Propranolol was perfused for 30 minutes at the time indicated by the arrows on the *x*-axis. Elevations in NE and decreases in blood pressure during drug perfusion periods (all doses) were significantly different from control values at $P < .05$, as determined by analysis of variance and Dunnet's *t*-test. (B) Correlation between propranolol-induced changes in mean arterial blood pressure and NE in CSF. The values plotted represent the maximal changes shown in (A).

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lation (*10*). Conceivably, the administration of propranolol into the brain results in an increased release of NE from noradrenergic neurons, which then activates central alpha-adrenergic receptors, leading to a fall in systemic arterial blood pressure. The present experiments were designed to test this hypothesis.

The ability of propranolol to release NE within the central nervous system was evaluated by determining the effect of centrally administered propranolol on the concentration of NE in cerebrospinal fluid (CSF). Since brain areas concerned with blood pressure regulation are densely innervated by noradrenergic nerves and are in close proximity to the brain ventricular areas, changes in noradrenergic nerve activity in these areas should be reflected in the concentration of NE in CSF (*11*). This assumption is supported by our observation that the NE-releasing drug tyramine increased NE in CSF dramatically (0.284 ± 0.114 to 24.702 ± 0.640 ng/ml) when perfused through the ventriculocisternal system of the dog brain. Similar changes in NE in the CSF have been reported following amphetamine treatment and central administration of angiotensin (*11, 12*).

Experiments were performed in mongrel dogs in which a ventriculocisternal perfusion system was established. The animals were anesthetized with sodium pentobarbital and the lateral ventricle was cannulated stereotaxically (coordinates: 16.0 mm anterior to bregma, 8.0 mm lateral to midline, 20.0 mm below the surface of the brain). A second cannula was inserted into the cisterna magna percutaneously. Ventriculocisternal perfusion (0.2 ml/min) with artificial CSF (*13*) was initiated, and the effluent was collected from the cannula in the cisterna magna at 15-minute intervals into chilled test tubes containing ascorbic acid. These were frozen at −70°C until being radioenzymatically assayed for NE (*14*). After a 90-minute control period, propranolol (1 to 50 μg/kg per minute) was added to the perfusate for 30 minutes. Drug-free CSF perfusion was then resumed and continued for an additional 120 minutes. Throughout the experiment, arterial pressure was monitored by a catheter in the femoral artery and heart rate was monitored by a tachograph triggered from lead 2 of an electrocardiograph.

The concentration of NE in CSF remained stable (0.100 to 0.300 ng/ml) during the drug-free perfusion period. The addition of *dl*-propranolol to the perfusate produced dose-dependent increases in NE and concomitant decreases in mean arterial blood pressure (Fig. 1A)

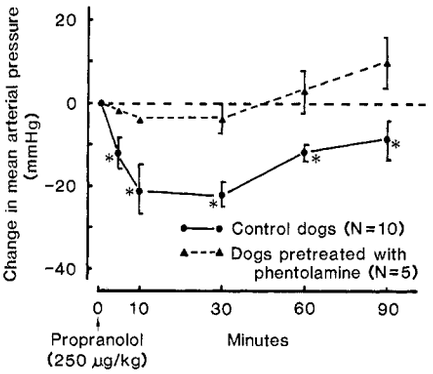

Fig. 2. Effect of the alpha-adrenergic blocking agent phentolamine (5 mg, intracisternally) on the hypotensive response to *dl*-propranolol (250 μg/kg). Propranolol was administered intracisternally at 0 minutes. Animals pretreated with phentolamine were given the agent 30 minutes before receiving propranolol. At 0 minutes mean arterial blood pressure was 127 ± 4 mmHg for dogs receiving propranolol alone and 106 ± 2 mmHg for pretreated dogs. Asterisks indicate significant differences at $P < .05$ (paired two-tailed *t*-test).

The lowest dose of propranolol (1 μg/kg per minute) decreased mean arterial pressure from 133 ± 1 to 113 ± 4 mmHg ($P < .05$) and increased the concentration of NE from 0.175 ± 0.036 to 0.432 ± 0.070 ng/ml ($P < .05$); the highest dose (50 μg/kg per minute) decreased mean arterial pressure from 144 ± 1 to 92 ± 13 mmHg ($P < .01$) and increased NE from 0.276 ± 0.009 to 7.438 ± 2.485 ng/ml ($P < .05$). At all doses the times at which the changes in NE began and peaked preceded those for blood pressure. In some animals an initial transient pressor response was also observed, but this effect was neither dose-dependent nor correlated with changes in NE. A highly significant correlation ($r = .94$, $P < .001$) was found between the increase in NE and the decrease in arterial pressure produced by propranolol (Fig. 1B).

It has been demonstrated that *d*-propranolol is equipotent with both the racemic mixture and the levorotatory isomer of the drug with respect to central hypotensive activity (*6, 7, 15*). Since the decrease in arterial pressure produced by propranolol may be the result of an increased release of NE from central neurons, it is important to determine whether *d*-propranolol also increases CSF NE. In four experiments *d*-propranolol (25 μg/kg per minute for 30 minutes) was administered into the ventriculocisternal system. During the control perfusion period, mean arterial pressure (114 ± 14 mmHg) and the concentration of NE in CSF (0.125 ± 0.012 ng/ml) remained stable. Addition of *d*-propranolol to the perfusate resulted in a maximum decrease in arterial pressure of 40 ± 2 mmHg accompanied by a 20-fold increase in NE (2.495 ± 0.797 ng/ml). These responses were not significantly different from those seen with the same dose of *dl*-propranolol.

Blockade of alpha-adrenergic receptors in the brain by phentolamine prevents the hypotension and bradycardia produced by the central administration of NE (*1, 2*). Since propranolol increases NE in CSF and this increase might be responsible for the hypotensive action of the drug, the effect of phentolamine pretreatment on the centrally mediated hypotensive action of propranolol was examined. Pentobarbital-anesthetized dogs were prepared as before for monitoring arterial pressure and heart rate. A 21-gauge, 1.5-inch-long stainless steel needle was inserted percutaneously into the cisterna magna for drug administration. Intracisternal injection of propranolol (250 μg/kg) produced a significant decrease in mean arterial pressure (Fig. 2). The onset of action was rapid (within 5 minutes), with the maximal effect occurring 30 minutes after injection. Pretreatment with phentolamine (5 mg, intracisternally) abolished the hypotension and bradycardia induced by propranolol (250 μg/kg).

Some investigators have suggested that the prolonged hypotensive effect seen after central administration of propranolol is due to blockade of central beta-adrenergic receptors (*16*). However, in this and other studies (*6, 7*), a hypotensive effect was observed following the administration of *d*-propranolol. Since the dextrorotatory isomer has only 1 to 2 percent of the beta-adrenergic blocking activity of the racemic mixture (*14*), the central actions of propranolol may be independent of an interaction with stereospecific beta-adrenergic receptors. Nor does the hypotension appear to be due to the local anesthetic action of the drug, since central administration of local anesthetics does not reduce blood pressure (*4, 7*).

Other studies have found that destruction of central catecholaminergic nerve terminals or depletion of catecholamines in the brain prevents the hypotensive effect of centrally administered propranolol (*6, 9*). These studies indicate that integrity of the central noradrenergic neuron is necessary for the hypotensive action of propranolol and support the hypothesis that the action is related to the effect of the drug on the noradrenergic nerve terminal. The present study also supports this hypothesis with the finding that the decrease in arterial pres-

sure produced by the central administration of propranolol is associated with an elevation in the concentration of NE in CSF. Furthermore, the high correlation between the hypotensive effects and the elevation in NE strongly suggests that the hypotensive effect produced by propranolol results from increased release or decreased reuptake of NE into central noradrenergic nerve terminals. That prior treatment with phentolamine prevents the hypotension is consistent with this hypothesis and suggests that the decrease in blood pressure is mediated through stimulation of central alpha-adrenergic receptors.

The possibility that the increased level of CSF NE results from a NE-releasing action of propranolol is supported by recent findings in other studies. Saelens *et al.* (*17*) found that propranolol enhances the spontaneous release of ^{3}H-labeled NE from adrenergic nerve endings in isolated guinea pig vas deferens. Daniell *et al.* (*10*) reported that intracoronary injection of propranolol induces the release of NE from adrenergic nerve endings in the canine heart. In both instances this effect of propranolol was not stereospecific.

In summary, our data support the idea that the hypotensive response to centrally administered propranolol results from an action of the drug to release NE, which stimulates central alpha receptors to decrease peripheral sympathetic nerve activity and lower arterial pressure. Although our study does not exclude a role for central beta receptors in the control of blood pressure, it does suggest that they do not play a major role in the hypotension seen after central administration of propranolol.

RANDALL L. TACKETT
JERRY G. WEBB
PHILIP J. PRIVITERA
Department of Pharmacology, Medical University of South Carolina, Charleston 29425

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Depolarization- and Ionophore-Induced Release of Octacosa Somatostatin from Stalk Median Eminence Synaptosomes

Abstract. *Species of somatostatin of higher molecular weight were present in the nerve terminals (synaptosomes) of ovine stalk median eminences and were released by depolarizing stimuli. One of these species was identified as the biologically active molecule octacosa somatostatin. Octacosa somatostatin appears therefore to be secreted into the hypothalamic-hypophyseal system, supporting the concept of a role for this peptide in regulating pituitary hormone secretion.*

Somatostatin is a tetradecapeptide originally isolated from ovine hypothalami as a result of its ability to inhibit growth hormone release (*1*). A number of immunoreactive species of somatostatin of higher molecular weight have been found in various tissues (*2*) and have been shown to have biological activity (*3, 4*). Recently a putative prohormonal form of somatostatin containing 28 amino acids (octacosa somatostatin) was isolated from pig intestine (*5*) and hypothalamus (*6*) and from sheep hypothalamus (*7*). This molecule has an extension of 14 amino acids at the NH_2-terminus of the somatostatin sequence. Amino acids 13 and 14 are the basic residues lysine and arginine, a characteristic tryptic cleavage point of many prohormones.

Unlike most prohormones, octacosa somatostatin exhibits substantial biological activity, having growth hormone release-inhibiting activity equal to (*6*) or greater than (*7*) that of somatostatin. Thus, octacosa somatostatin may be an active regulator of growth hormone secretion, and somatostatin may be a biologically active fragment (*7*). The presence of biologically active octacosa somatostatin in tissue does not indicate that the peptide is secreted and thus performs a biological role. We have now demonstrated that octacosa somatostatin is contained in nerve terminals (synaptosomes) isolated from ovine stalk median eminences, and that the peptide is released by a depolarizing concentration of KCl (100 m*M*) and by the calcium ionophore A23187. These findings suggest that the peptide is secreted into the hypothalamic-hypophyseal portal system in vivo and support the concept of a biological role for octacosa somatostatin.

Stalk median eminences were dissected from sheep hypothalami and homogenized. Synaptosomes were purified by differential and discontinuous Ficoll density gradient ultracentrifugation (*8*). Electron microscopy showed that the preparation was composed predominantly of intact synaptosomes. The synaptosomes actively sequestered [^{3}H]noradrenaline, γ-[^{3}H]aminobutyric acid, and [^{14}C]acetylcholine at 37°C, while uptake did not occur at 4°C. Intactness of synaptosomes was demonstrated by the high proportion of occluded lactate dehydrogenase, since 82 percent of the total activity was released on exposure to hypoosmotic medium.

Extraction of synaptosomes with 0.2*N* acetic acid and separation of the components on Sephadex G-25 (fine) revealed a major somatostatin immunoreactive peak, which eluted with synthetic somatostatin-14, and two earlier-eluting immunoreactive peaks (Fig. 1A). The apparent molecular weights of the two peaks were 5000 or larger, and 3000. The 3000-dalton immunoreactive peak eluted with synthetic octacosa somatostatin (*6, 9*).

Stimulation of synaptosomes with 100 m*M* KCl or the ionophore A23187 released immunoreactive somatostatin into the medium. The immunoreactive material was fractionated on Sephadex G-25 (fine) into three immunoreactive peaks that eluted in the positions of somatostatin-14, octacosa somatostatin, and the species of 5000 daltons or larger (Fig. 1B). In contrast, luteinizing hormone–releasing factor was released from these

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