

## Short Communication

# 5-Hydroxytryptamine: Source of Activator Calcium in Human Basilar Arteries

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**SUMMARY** We performed experiments in human cerebral arteries to determine the source of activator calcium during contractions induced by 5-hydroxytryptamine. Rings of human basilar artery obtained at autopsy were mounted for isometric tension recording in organ baths filled with a physiological salt solution. Contractile responses to 5-hydroxytryptamine were virtually abolished in  $\text{Ca}^{++}$ -free solution, and inhibited significantly by nimodipine. In both cases, the depression of the response to 5-hydroxytryptamine was comparable to that seen when KCl was used to contract the vessels. These experiments demonstrate that 5-hydroxytryptamine mediates contraction of the smooth muscle in human basilar artery by increasing membrane permeability to extracellular calcium.

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ALTHOUGH 5-HYDROXYTRYPTAMINE is a potent constrictor of cerebral arteries from numerous animal species,<sup>1-3</sup> its mechanism for making  $\text{Ca}^{++}$  available to activate the contractile proteins appears to be species-dependent. While contractions to 5-hydroxytryptamine in dog and rabbit basilar arteries depend upon the influx of extracellular  $\text{Ca}^{++}$ ,<sup>1,3</sup> in the bovine basilar artery they are not associated with transmembrane  $\text{Ca}^{++}$  influx,<sup>2</sup> suggesting that the ion is made available by mobilization of intracellular stores. The availability of calcium entry blockers with a relative specificity for cerebrovascular smooth muscle,<sup>4-6</sup> provides a method for determining the  $\text{Ca}^{++}$ -dependency of the contractile responses to neurohumoral mediators implicated in clinical conditions such as cerebral vasospasm. In view of the role that 5-hydroxytryptamine may play in such pathological situations,<sup>7-9</sup> we performed experiments to determine the source of activator  $\text{Ca}^{++}$  during 5-hydroxytryptamine-induced contractions in isolated human basilar arteries.

### Methods

Basilar arteries were removed from cadavers (ages 44–79 years) donated to the Mayo Foundation for the purpose of medical science. The vessels were obtained between 4 and 23 hours after death. After removal, the arteries were cleaned of connective tissue and cut into 3 mm rings. The rings were placed in organ chambers (25 ml volume) and attached to strain gauges (Statham UC2) for isometric tension recording. Experiments were performed at 37°C in a physiological salt solution (composition, mM: NaCl, 118.3; KCl, 4.7;  $\text{MgSO}_4$ ,

1.2;  $\text{KH}_2\text{PO}_4$ , 1.2;  $\text{CaCl}_2$ , 2.5;  $\text{NaHCO}_3$ , 25; CaEDTA, 0.026; and glucose, 11.1; control solution) aerated with a 95%  $\text{O}_2$ –5%  $\text{CO}_2$  gas mixture. Phentolamine ( $10^{-5}\text{M}$ ) and propranolol ( $5 \times 10^{-6}\text{M}$ ) were maintained in the bath solution to provide alpha- and beta-adrenergic blockade, respectively. The rings were placed at the optimal point of their length-active tension relationship using KCl (40 mM), and allowed to equilibrate for 30 minutes. Average basal tension before each experiment began was 2.3 grams.

In rings from the same artery, initial dose-response curves to 5-hydroxytryptamine ( $10^{-10}$  to  $10^{-4}\text{M}$ ) or KCl (10 to 40 mM) were obtained. Contractile responses to 5-hydroxytryptamine and KCl were compared, since KCl-induced contractions are known to depend upon the influx of extracellular  $\text{Ca}^{++}$ .<sup>10-12</sup> After washout of the agonists and a one hour equilibration period, 2 sets of experiments were performed in different rings: (a) arteries were exposed to  $\text{Ca}^{++}$ -free solution containing 2 mM EGTA for 10 min, and dose-response curves to 5-hydroxytryptamine or KCl were repeated in the absence of  $\text{Ca}^{++}$  or (b) the  $\text{ED}_{50}$  (concentration of agonist causing one-half maximal contraction) for 5-hydroxytryptamine and KCl was used to contract the arteries; after stable contractions occurred, nimodipine ( $10^{-10}$  to  $10^{-7}\text{M}$ ) was added to the bath solution in increasing concentrations to obtain a dose-relaxation response. In all cases, parallel control experiments were performed to rule out time- or solvent-induced changes in tension.

The drugs used were: 5-hydroxytryptamine (serotonin; Sigma), nimodipine (Miles Laboratories), phentolamine (Ciba) and propranolol (Sigma). Nimodipine was dissolved in polyethylene glycol, and added directly to the bath solution in 0.05 ml aliquots. Other drugs were dissolved in distilled water and added in 0.1 ml aliquots. The concentrations are expressed as final bath concentrations. The KCl concentration of the physiological salt solution was altered by equimolar replacement of NaCl. Increases in KCl concentra-

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tions were achieved by overflowing the organ chamber with high  $K^+$  solutions kept in a reservoir at  $37^\circ\text{C}$ .

For statistical analysis, Student's *t*-test for paired observation was used. *P* values less than 0.05 were considered to be statistically significant.

### Results

In rings from 6 arteries, dose-response curves to 5-hydroxytryptamine and KCl were performed in control solution ( $2.5\text{ mM Ca}^{++}$ ) and in  $\text{Ca}^{++}$ -free solution following 10 min exposure to  $\text{Ca}^{++}$ -free solution containing  $2\text{ mM EGTA}$ . In the absence of extracellular  $\text{Ca}^{++}$ , the two agonists did not cause significant increases in tension (fig. 1).

When contractions were obtained with 5-hydroxytryptamine ( $4 \times 10^{-6}\text{ M}$ ) or KCl ( $20\text{ mM}$ ), the addition of increasing concentrations of nimodipine caused a concentration-dependent relaxation. At  $10^{-9}\text{ M}$ , nimodipine caused significant relaxation of contractions induced by 5-hydroxytryptamine. At the higher concentrations of antagonist used ( $10^{-8}$  and  $10^{-7}\text{ M}$ ), contractions caused by 5-hydroxytryptamine and KCl were markedly inhibited (fig. 2).

### Discussion

Previous investigators have shown that 5-hydroxytryptamine contracts human cerebral arteries.<sup>13-15</sup> In human pial arteries, this contraction can be attenuated by the calcium channel blocking agent nifedipine.<sup>16</sup> The present experiments concur with this report, and demonstrate that in the human basilar artery, 5-hydroxytryptamine mediates contraction by increasing membrane permeability to extracellular calcium. In this respect, human basilar arteries resemble canine and rabbit,<sup>1,3</sup> but not bovine<sup>2</sup> cerebral arteries. As in all other blood vessels tested so far,<sup>10-12</sup> the contractile response to KCl was also dependent upon extracellular

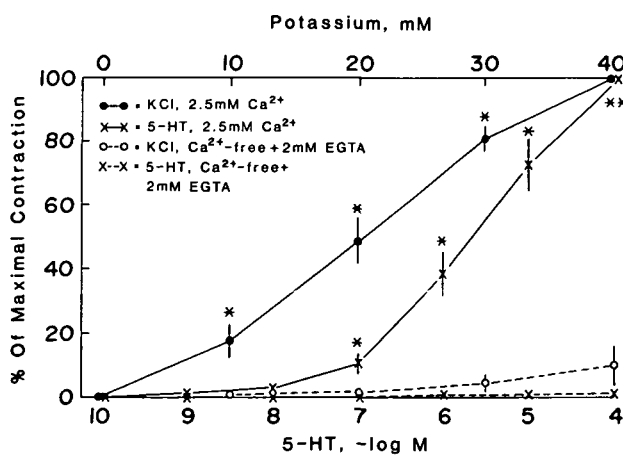


FIGURE 1. Effect of incubation in  $\text{Ca}^{++}$ -free solution on the concentration-contraction curves to KCl and 5-hydroxytryptamine (5-HT) in isolated human basilar arteries. Data shown as means ( $n = 6$ )  $\pm$  SEM, and expressed as percent of the contraction obtained with the largest concentration of each agonist. \* = the effect of  $\text{Ca}^{++}$ -free +  $2\text{ mM EGTA}$  solution is statistically significant.

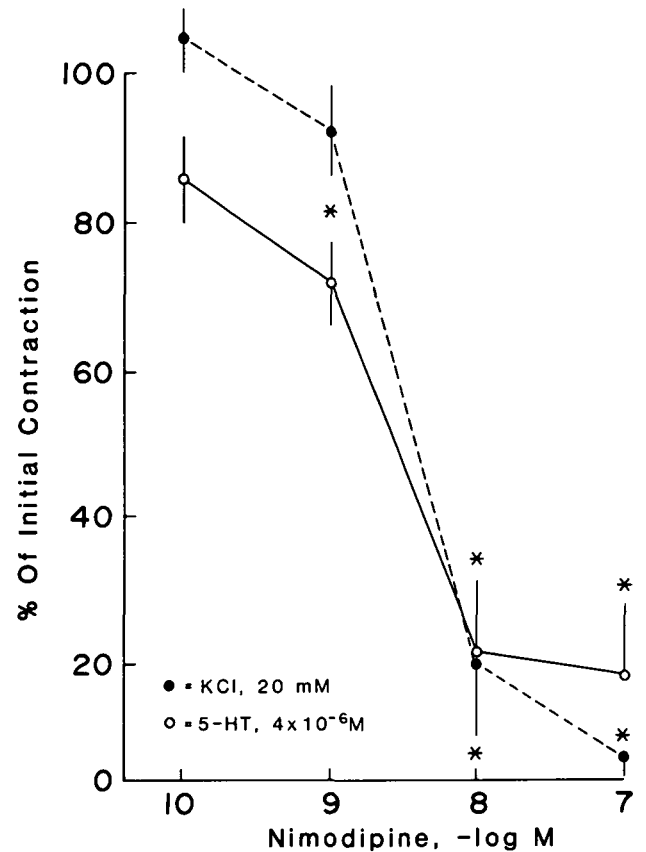


FIGURE 2. Effect of increasing concentrations of the  $\text{Ca}^{++}$  entry blocker nimodipine on contractions of human basilar arteries evoked by the  $\text{ED}_{50}$  of either KCl or 5-hydroxytryptamine (5-HT). Data shown as means ( $n = 6$ )  $\pm$  SEM, and expressed as percent of the initial contraction. \* = the effect of nimodipine is statistically significant.

$\text{Ca}^{++}$ . The observation that both  $\text{Ca}^{++}$ -free solution and nimodipine also markedly depressed responses to 5-hydroxytryptamine, suggests that mobilization of intracellular  $\text{Ca}^{++}$  stores must play at best a minor role in the contractile response to the monoamine.

It has been reported that calcium channel blocking agents increase cerebral blood flow in animal models<sup>3</sup> and humans<sup>17</sup> after acute ischemic stroke, and can prevent or alleviate cerebral vasospasm.<sup>18,19</sup> High extracellular  $K^+$  concentrations and 5-hydroxytryptamine-induced constriction, respectively, have been implicated as possible spasmogenic agents in these pathologies.<sup>3,7-9</sup> The present study predicts that calcium channel blocking agents should be particularly effective to treat increased cerebrovascular tone caused by these agents.

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