Synaptic Potentials in Sympathetic Ganglia: Are They Mediated by Cyclic Nucleotides?

Abstract. *The hypothesis that cyclic nucleotides are intracellular second messengers mediating the generation of synaptic potentials was studied in the sympathetic ganglia of the bullfrog. Synaptic potentials and the effect of administering cyclic nucleotides and agents which affect cyclic nucleotide metabolism were recorded by the sucrose gap technique. The administration of adenosine 3',5'-monophosphate (cyclic AMP), guanosine 3',5'-monophosphate (cyclic GMP), or several of their derivatives produced little or no change in membrane potential. Prostaglandin E_1 did not block the generation of postsynaptic potentials. Theophylline produced membrane effects that were different from those associated with postsynaptic potential generation; it also reduced the slow excitatory postsynaptic potential (EPSP) and potentiated the slow inhibitory postsynaptic potential (IPSP). The administration of papaverine, however, reduced both the slow EPSP and the slow IPSP. Although synaptic stimulation increases both cyclic GMP and cyclic AMP in these neurons, these results raise the possibility that these cyclic nucleotides may have functional roles other than mediation of synaptic potentials.*

The molecular basis of synaptic potential generation in nervous tissue is of considerable neurobiological interest. In several studies in recent years sympathetic ganglia have been used to investigate this problem. There are two major advantages to this preparation. First, several types of synaptic potentials can be elicited which are amenable to analysis by electrophysiological techniques. These include long-duration excitatory and inhibitory postsynaptic potentials (the slow EPSP and the slow IPSP) which, because of their membrane properties, can provide long-lasting mechanisms of synaptic integration and control of neuronal interaction (*1, 2*). Second, sympathetic ganglia offer a relatively homogeneous population of neurons for analysis by biochemical techniques. It is possible to stimulate preganglionic nerve fibers and to correlate the resulting postsynaptic biochemical changes with the physiology and pharmacology of the postsynaptic potentials (PSP'S). Such an investigation of rabbit superior cervical sympathetic ganglion revealed that stimulation of the preganglionic nerve fibers increased the concentration of adenosine 3',5'-monophosphate (cyclic AMP) in the postganglionic neurons, and pharmacological studies showed that agents affecting the slow IPSP also affected the cyclic AMP increase (*3*).

Three lines of electrophysiological evidence suggested that the production of the slow IPSP was mediated by cyclic AMP (*4*): (i) the administration of cyclic AMP or dibutyryl cyclic AMP produced a hyperpolarization of the postganglionic neurons; (ii) the slow IPSP was blocked by prostaglandin E_1 (PGE_1), a substance which had been presumed to inhibit adenylate cyclase, the enzyme catalyzing the synthesis of cyclic AMP; and (iii) the slow IPSP was potentiated by theophylline, a drug which inhibits phosphodiesterase, the enzyme which breaks down cyclic nucleotides. In addition, since the administration of guanosine 3',5'-monophosphate (cyclic GMP) or dibutyryl cyclic GMP produced a depolarization of the postganglionic neurons, it was also proposed that cyclic GMP may mediate the slow EPSP [see also (*5*)].

In the study reported here we further investigated the possibility that cyclic nucleotides mediate the slow postsynaptic potentials, using sucrose gap recording to study sympathetic ganglia of the bullfrog. This preparation was selected for several reasons. First, the slow EPSP and the slow IPSP can be elicited in separate cell types by stimulation of separate preganglionic nerves (*6*). Second, the ionic membrane mechanisms involved in generating the slow EPSP and the slow IPSP have been investigated most extensively in these sympathetic ganglia (*1, 2, 6-8*), making it potentially possible to determine whether the administration of a cyclic nucleotide produces permeability changes identical to those involved in generating the slow PSP's. Third, presynaptic stimulation increases both cyclic AMP and cyclic GMP in these ganglia (*9*). In this study we found few, if any, effects of administering either cyclic AMP, cyclic GMP, or a number of their derivatives; nor did PGE_1 block the generation of PSP's. In addition, we found that the

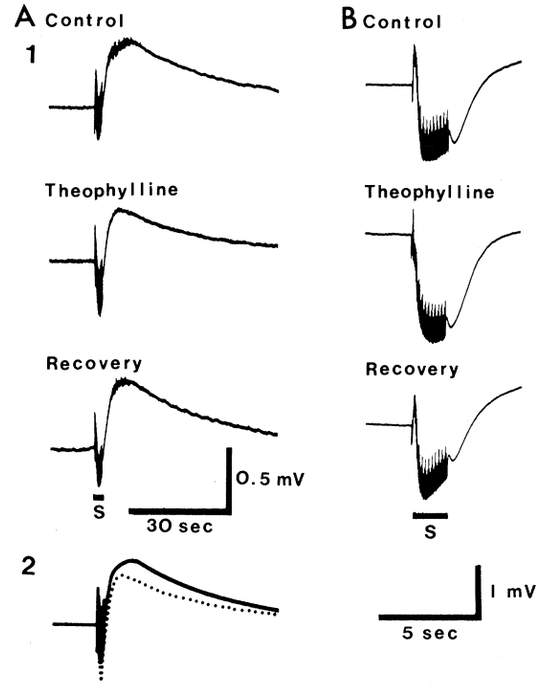

Fig. 1. Effect of theophylline on (A) the slow EPSP and (B) the slow IPSP. (A1) The upper trace shows the control record of the slow EPSP produced by repetitive supramaximal stimulation of the sympathetic chain rostral to the seventh ganglion at a frequency of 100 Hz, the middle trace the effect of theophylline on the slow EPSP 35 minutes after the start of superfusion with Ringer solution containing 5 m*M* theophylline, and the lower trace the recovery record of the slow EPSP 40 minutes after the start of theophylline washout. (A2) Tracing of records in (A1): control (solid line) and theophylline (dotted line) effects on slow EPSP's. Note the reduction of slow EPSP amplitude; the amplitude in theophylline averaged 81 percent ($N = 17$) of that in the control. (B) The upper trace shows the control record of the slow IPSP produced by repetitive supramaximal stimulation of the eighth spinal nerve at a frequency of 50 Hz, the middle trace the effect of theophylline on the slow IPSP 70 minutes after the start of superfusion with Ringer solution containing 5 m*M* theophylline, and the lower trace the recovery record of the slow IPSP 85 minutes after the start of theophylline washout. The slow IPSP amplitude in theophylline averaged 170 percent ($N = 17$) of that in the control. In most experiments, the maximal effect of theophylline on PSP generation and the maximal recovery after beginning theophylline washout occurred in 30 to 60 minutes. The period of presynaptic stimulation is indicated by the bar labeled *S*. The records are from a rectilinear pen recorder (Brush model 280). Experiments were performed on the ninth or tenth paravertebral sympathetic ganglion of the bullfrog, using the sucrose gap recording technique. To prevent activation of nicotinic receptors by acetylcholine or MCh, (B) *d*-tubocurarine (70 μM) or (A) nicotine (30 μM) was used as a nicotinic antagonist; essentially the same results were obtained with either *d*-tubocurarine or nicotine.

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neuronal effects of theophylline are complex and are different from those of another phosphodiesterase inhibitor, papaverine. The results indicate that further investigation is needed to establish whether cyclic nucleotides mediate the generation of synaptic potentials in sympathetic ganglia.

The first electrophysiological observation supporting cyclic nucleotide mediation of slow PSP's in rabbit superior cervical ganglion was the response to the administration of the cyclic nucleotide (*4*). In the present investigation, in three preparations, we observed a depolarization associated with the administration of cyclic GMP (1 m*M*). However, these observations were not repeatable, since in 80 other tests on 20 preparations no consistent response to cyclic GMP or its derivatives has been observed. In addition, no response has been observed to the administration of cyclic AMP (1 m*M*). This lack of response might be due to low permeability of the membrane to these cyclic nucleotides or to enzymatic degradation. In an attempt to overcome these difficulties, we also used the dibutyryl (DB), 8-bromo (8-Br), and 8-parachlorophenylthio (8-PCPT) derivatives, which have greater membrane permeability or are more resistant to phosphodiesterase action, or both (*10, 11*). In addition, the 8-substituted derivatives are especially potent in activating protein kinase, the enzyme which mediates the actions of cyclic nucleotides (*5, 11, 12*). In several experiments, theophylline was used to inhibit phosphodiesterase (*13*), yet even in the presence of 5 m*M* theophylline, we did not observe responses to cyclic AMP, cyclic GMP, or their 8-Br or 8-PCPT derivatives. These results obtained with the sucrose gap technique are supported by similar negative results obtained by using intracellular recording combined with extracellular or intracellular application of cyclic nucleotides (*14*).

The second line of evidence supporting cyclic nucleotide mediation of the slow PSP's in rabbit sympathetic ganglia was inhibition of the PSP by PGE_1 (10^{-8} to $10^{-6}M$) (*4*). In the present investigation, PGE_1 in the concentration range 10^{-8} to $10^{-4}M$ did not block PSP generation.

The third line of evidence supporting cyclic nucleotide mediation of a PSP in rabbit sympathetic ganglia was potentiation by theophylline (*4*). Figure 1A illustrates the effect of theophylline on the slow EPSP in the present experiments; it can be seen that the amplitude of the slow EPSP was reduced by theophylline (*15*). A reduction of slow EPSP amplitude by theophylline (5 m*M*) was observed in 14 of 17 experiments. Figure 1B illustrates the effect of theophylline on the slow IPSP. Theophylline increased slow IPSP amplitude in 16 of 17 experiments. Since theophylline can affect transmitter release (*16*), we also tested the effect of theophylline on the response to methacholine (MCh), an agonist that directly activates the postsynaptic receptors involved in generating the slow PSP's (*8, 17*). The effects of theophylline on the MCh-induced hyperpolarization and depolarization were similar to its effects on the slow IPSP and the slow EPSP, respectively (Fig. 2A). On the other hand, the administration of papaverine ($10^{-4}M$), a nonmethylxanthine phosphodiesterase inhibitor 10 to 1000 times more potent than theophylline (*18*), reduced the amplitude of the slow IPSP, the slow EPSP, or the MCh responses in six of seven preparations (*19*).

Administration of theophylline also had effects on the membrane of the neurons, producing a depolarization of the membrane potential, a decrease in membrane input resistance, and a prolongation of the spike after-hyperpolarization (AH) (*20, 21*). Since this potentiation of the spike AH is abolished by the removal of extracellular calcium (*20*), we were interested in testing the effect of Ca removal on the theophylline potentiation of the MCh hyperpolarization (*22*). Figure 2B shows that in Ca-free Ringer solution theophylline greatly increased the amplitude of the MCh hyperpolarization and virtually abolished the MCh depolarization (*23*).

The effects of theophylline and papaverine observed in the present experiments were complex. The membrane effects of theophylline itself were different from the membrane changes associated with slow PSP generation (*2, 7, 8*). The reduction in slow EPSP amplitude produced by theophylline and papaverine is the opposite of what would be expected if its generation is cyclic nucleotide-mediated. On the other hand, the potentia-

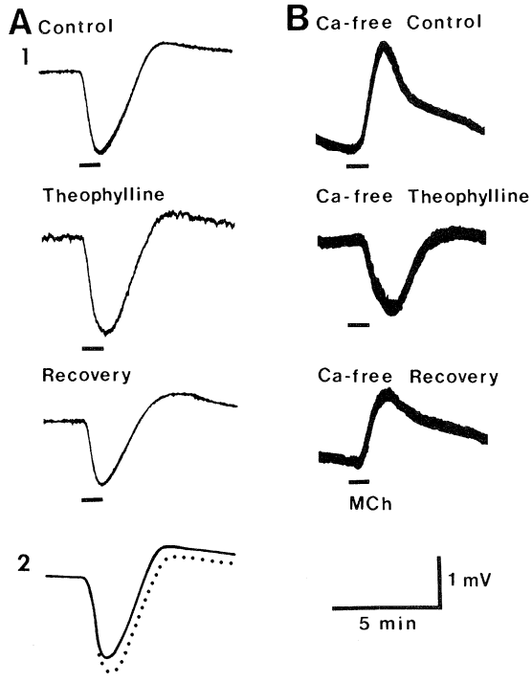

Fig. 2. Effect of theophylline on MCh responses in (A) normal and (B) Ca-free Ringer solution. (A1) The upper trace shows the control record of initial MCh hyperpolarization and subsequent MCh depolarization, which represent activation of postsynaptic receptors involved in generating the slow IPSP and the slow EPSP, respectively; the middle trace, the effect of theophylline on MCh responses 1 hour and 55 minutes after the start of superfusion with Ringer solution containing 5 m*M* theophylline; and the lower trace the recovery record taken 2 hours and 20 minutes after the start of theophylline washout. (A2) Tracing of records in (A1): control (solid line) and theophylline (dotted line) effects on MCh responses. Note the potentiation of MCh hyperpolarization and reduction of MCh depolarization. During the action of theophylline, the MCh hyperpolarization amplitude averaged 184 percent (N = 9) and the MCh depolarization amplitude averaged 84 percent (N = 14) of the control amplitude. (B) The upper trace shows the control record of MCh responses in Ca-free Ringer solution. The preparation was superfused with Ca-free Ringer solution for 3 hours and 20 minutes before the control record was made. With the sucrose gap technique, the MCh hyperpolarization and the MCh depolarization are to a large extent superimposed; in this experiment, the large MCh depolarization obscures the MCh hyperpolarization. In other experiments both were observed in Ca-free Ringer solution. The middle trace shows the effect of theophylline on MCh responses in Ca-free Ringer solution 1 hour and 15 minutes after the start of superfusion with Ringer solution containing 5 m*M* theophylline. Note the large potentiation of MCh hyperpolarization and disappearance of MCh depolarization. The bottom trace shows the recovery record of MCh responses in Ca-free Ringer solution 1 hour and 55 minutes after the start of theophylline washout. The recordings were made as in Fig. 1. Methacholine administration is indicated by the bar labeled *MCh*. The nicotinic antagonist was *d*-tubocurarine (70 μM) in both (A) and (B). The ganglion was continuously superfused with oxygenated Ringer solution of the following composition: NaCl, 100 m*M*; KCl, 2 m*M*; $CaCl_2$, 1.8 m*M*; tris-HCl, 16 m*M* (*p*H 7.2); and glucose, 1 g/liter. Calcium-free Ringer solution had the same composition except that $CaCl_2$ was omitted.

tion of the slow IPSP by theophylline is consistent with the possibility that the slow IPSP is mediated by a cyclic nucleotide. This effect, however, was not produced by the administration of papaverine (*19*). This raises the question of whether the potentiation of the slow IPSP by theophylline is a direct result of phosphodiesterase inhibition. It should be noted that this potentiation can be explained by other mechanisms. For example, the theophylline-induced increase in resting membrane permeability (*24, 25*) would result in an increased resting sodium influx. Since there is evidence that the slow IPSP is generated by inactivation of resting Na permeability (*2, 8*), such inactivation of an increased resting Na influx would be expected to result in the generation of a larger slow IPSP.

Theophylline and other methylxanthines are known to affect Ca metabolism in several tissues (*26*). In the neurons studied in the present investigation, theophylline prolongs the duration of the spike AH (*20*). Since Ca removal abolishes this potentiation (*20*), but does not abolish the theophylline potentiation of the MCh hyperpolarization, it is unlikely that the potentiation of the slow IPSP involves a Ca-sensitive permeability change similar to that in the spike AH. On the other hand, the effect of Ca-free Ringer solution on the theophylline potentiation of the MCh responses indicates that calcium ions affect this interaction (*27*). Further investigation is needed to clarify the role of Ca in the generation of these responses. In several tissues, the accumulation of cyclic GMP is dependent on the presence of extracellular calcium ions (*28*). In the present experiments, the observation that both the MCh hyperpolarization and the MCh depolarization can be elicited in Ca-free Ringer solution suggests that cyclic GMP may not be involved in the electrogenesis of these slow potentials. However, it is not clear whether MCh stimulation of cyclic GMP accumulation is Ca-dependent in this tissue.

In these experiments, the administration of cyclic nucleotides did not mimic the electrogenesis of the slow PSP's, nor did PGE_1 block their generation (*29*). These data raise questions about cyclic nucleotide mediation of the slow PSP's. Such negative results should be viewed with caution, however, since there might be factors in the ganglion which prevent certain substances from reaching sites necessary to produce responses. The possibility that the cyclic nucleotides were hydrolyzed more rapidly than they penetrated the cells was minimized by using 8-substituted derivatives, which are both resistant to hydrolysis by phosphodiesterase and especially potent in activating protein kinase (*11, 12*). The combination of these derivatives with a phosphodiesterase inhibitor makes the possibility of rapid hydrolysis of these compounds most unlikely.

Several of the results reported here differ from those of previous observations on rabbit sympathetic ganglia (*4*), which provided the electrophysiological evidence supporting the proposal that cyclic nucleotides mediate the generation of slow PSP's; the reasons for these differences are not clear. The possibility that species variability may account for these differences is not supported by recent reports of several similar observations on rat and rabbit sympathetic ganglia (*30*). The electrophysiological properties of responses to cyclic nucleotides have been found to differ from the responses to neurotransmitters in several tissues (*25, 30, 31*). In addition, it has recently been demonstrated that cyclic nucleotide levels can be elevated in sciatic nerve without affecting membrane potential or action potential generation (*32*). Viewed in the context of these studies, the results reported here raise the possibility that the postsynaptic increases in cyclic nucleotide concentration resulting from presynaptic stimulation may have functional roles other than mediation of postsynaptic potential generation.

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22. Since the removal of extracellular Ca prevents the release of synaptic transmitter and thus blocks the slow PSP's, it was necessary to test the effect of Ca removal on the MCh responses.
23. Because of the temporal superposition of the MCh hyperpolarization of C cells and MCh depolarization of B cells in sucrose gap recording, a quantitative assessment of the effects of Ca-free Ringer solution on the separate responses will require intracellular recording from individual B and C cells.
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