Dual Effects of Theophylline on Spontaneous Transmitter Release from Frog Motor Nerve Terminals

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Alkylxanthine drugs, such as theophylline, block adenosine receptors, inhibit phosphodiesterases and other enzymes, and cause the release of calcium from intracellular stores. Adenosine receptor blockade occurs at low micromolar concentrations of the drugs, while other effects occur in the millimolar concentration range.

The effects of theophylline were tested on spontaneous transmitter release at the frog cutaneous-pectoris neuromuscular junction (NMJ). A change in the frequency, but not the amplitude, of miniature endplate potentials (mepps) was interpreted as a change in spontaneous transmitter release. In normal Ringer's, theophylline, at concentrations of 100 μ M and 1 mM, enhanced spontaneous transmitter output, while at 10 μ M, theophylline had no consistent effect on spontaneous release. In contrast, theophylline produced dual effects on mepp frequency in hyperosmotic Ringer's. At 10 μ M, theophylline depressed mepp frequency, while, at 100 μ M and 1 mM, theophylline increased mepp rate.

Since low micromolar concentrations of theophylline depressed spontaneous transmitter release, this action may result from adenosine receptor blockade and inhibition of a tonic, stimulatory effect of adenosine. This hypothesis was supported by the following experimental results: (1) Micromolar concentrations of theophylline reversed the effects of applied adenosine on neuromuscular transmission. (2) The inhibitory effect of theophylline was mimicked by 2 other alkylxanthines, 8-phenyltheophylline and 8-p-sulfophenyltheophylline. These drugs may be more specific adenosine receptor antagonists than theophylline. (3) The inhibitory effect of theophylline was mimicked by adenosine deaminase, an enzyme that breaks down and inactivates adenosine. (4) The depressant action of theophylline was masked by the addition of adenosine deaminase to the hyperosmotic Ringer's.

Application of adenosine to the frog NMJ reduces spontaneous transmitter output. Yet, theophylline, in hyperosmotic Ringer's, depressed mepp frequency probably by

adenosine receptor blockade and inhibition of the effects of endogenous adenosine. This paradox may be explained if adenosine has more than one action on spontaneous transmitter release from frog motoneurons.

The alkylxanthine, theophylline, affects the activity of excitable cells by several different mechanisms. At micromolar concentrations, theophylline blocks adenosine receptors (Sattin and Rall, 1970; Smellie et al., 1979; Daly et al., 1981), while, at millimolar concentrations, theophylline may depress the activity of several enzymes, including phosphodiesterases (Butcher and Sutherland, 1962; Fain et al., 1972; Fredholm et al., 1978; Smellie et al., 1979), 5'-nucleotidase (Tsuzuki and Newburgh, 1975; Fredholm et al., 1978), and alkaline phosphatase (Fredholm et al., 1978; Croce et al., 1979). In addition, millimolar concentrations of methylxanthines may cause the release of calcium from intracellular stores (Bianchi, 1968).

Theophylline enhances acetylcholine (ACh) release from vertebrate motoneurons (Goldberg and Singer, 1969; Ginsborg and Hirst, 1972; Wilson, 1974; Ribeiro and Sebastião, 1987). This effect has been attributed both to phosphodiesterase inhibition (Goldberg and Singer, 1969; Wilson, 1974) and adenosine receptor blockade (Ginsborg and Hirst, 1972; Ribeiro and Sebastião, 1987). Since application of adenosine usually depresses transmitter release from motor nerve terminals (Ginsborg and Hirst, 1972; Ribeiro and Walker, 1975; Brânisteanu et al., 1979; Silinsky, 1984), theophylline may enhance ACh output by blocking a tonic, depressant effect of endogenous adenosine.

The concentrations of theophylline that have been studied at the vertebrate neuromuscular junction (NMJ) have been in the millimolar or high micromolar concentration range (Goldberg and Singer, 1969; Ginsborg and Hirst, 1972; Wilson, 1974; Ribeiro and Sebastião, 1987). At such high concentrations, the drug may affect neuromuscular transmission by more than one mechanism. If theophylline enhances transmitter release by adenosine receptor blockade, its effects may be seen at micromolar concentrations (Smellie et al., 1979; Daly et al., 1981). If, however, the drug increases ACh output by inhibition of phosphodiesterase or other enzymes or by causing the release of intracellular calcium, its effects may be observed only at millimolar concentrations (Bianchi, 1968; Tsuzuki and Newburgh, 1975; Fredholm et al., 1978; Smellie et al., 1979).

I have studied the effects of theophylline on spontaneous transmitter release from frog motoneurons. In normal Ringer's, theophylline, at 10 and 20 μ M, produced no consistent effect on spontaneous transmitter output, while, at concentrations equal to or greater than 100 μ M, theophylline enhanced spontaneous ACh release. In contrast, in hyperosmotic Ringer's solution,

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Table 1. Changes in mepp frequency produced by theophylline in normal and hyperosmotic Ringer's

Theophylline dose	Percentage change in mepp frequency			
	Normal Ringer's	Mean $\pm$ SEM	Hyperosmotic Ringer's	Mean $\pm$ SEM
10 μ M	-22		-44 ^a	
	+0.7		-42 ^a	
	+7		-29 ^a	
	+42 ^a	+6.9 $\pm$ 13.2	-19	-33.5 $\pm$ 5.9
20 μ M	-17		-21	
	-11		-12	
	+25	-1.0 $\pm$ 13.1	+30 ^a	+12.5 $\pm$ 17.4
100 μ M	+45		+26 ^a	
	+53 ^a		+68 ^a	
	+55 ^a		+77 ^a	
	+62 ^a	+53.8 $\pm$ 3.5		+57.0 $\pm$ 15.7
1 mM	+59 ^a		+80 ^a	
	+71 ^a		+102 ^a	
	+73 ^a	+67.7 $\pm$ 4.4	+128 ^a	+103.3 $\pm$ 13.9

At concentrations of 10 and 20 μ M, theophylline did not produce a consistent effect on mepp frequency in normal Ringer's, but, at 10 μ M, theophylline consistently depressed mepp frequency in hyperosmotic Ringer's. At 100 μ M and 1 mM, theophylline enhanced mepp frequency both in normal and hyperosmotic Ringer's. A 2-sample Student's *t* test was used to compare in each preparation mean mepp frequencies in the presence and absence of theophylline.

^a *p* $\leq$ 0.05.

theophylline produced dual effects on spontaneous transmitter release. At millimolar concentrations (0.1 and 1.0 mM), theophylline enhanced spontaneous transmitter release from frog motoneurons, while, at low micromolar concentrations (10 μ M), theophylline depressed spontaneous transmitter output. This depressant effect of theophylline was mimicked by the enzyme adenosine deaminase and may therefore result from blockade of adenosine receptors and inhibition of the effects of endogenous adenosine (Daly, 1982). However, application of adenosine to the frog NMJ also reduces spontaneous transmitter output (Ribeiro and Walker, 1975; Brănisteanu et al., 1979; Silinsky, 1984). These results suggest that adenosine both stimulates and inhibits spontaneous transmitter release from frog motoneurons. A preliminary report of this research has appeared (Barry, 1986a).

Materials and Methods

The cutaneous-pectoralis nerve-muscle preparation was isolated from small frogs (*Rana pipens*, 1½ to 2½ inches, Kons Scientific, Germantown, WI). The nerve-muscle preparation was placed in a recording dish and continually perfused with frog Ringer's (115 mM NaCl, 1.8 mM CaCl₂, 2 mM KCl, 5 mM glucose, 5 mM Tris HCl, pH 7.2). Hyperosmotic Ringer's solution was made by adding 24 mM sucrose to the Ringer's. Intracellular recordings of miniature endplate potentials (mepps) were made with 20–40 MΩ glass electrodes placed in the endplate region of the muscle fiber. Recordings were amplified on a Biotronic AM-4 amplifier, displayed on a Tektronix 5113 oscilloscope, and stored on an Indec IR-2 data recorder or photographed on a Grass Kymograph camera. Microelectrodes were usually filled with 3 M KCl. In most cases, muscle resting potentials of about -95 mV could be obtained for several hours. During the winter, however, the resting potential of the muscle fibers tended to depolarize over time. The problem was corrected by filling the microelectrodes with a 250 mM KCl solution as opposed to a 3 M KCl solution. This change in the filling solution reduced the muscle resting potential by about 10 mV but had no other effects on the experimental results. Recordings were made at 18–21°C. Bath temperature

was controlled by a peltier device. In any one experiment, the temperature did not vary by more than 1°C.

A change in mepp frequency that was not accompanied by a change in mepp size was interpreted as a change in spontaneous transmitter release (Katz, 1969). Mean mepp frequency was measured by counting the number of mepps occurring during 30–60 consecutive 10 sec recording periods in each solution and averaging these values. Mean mepp frequencies obtained from one preparation recorded in different solutions were compared using the 2 sample Student's *t* test. Since the effects of all the drugs tested were reversible, mean mepp frequencies in the presence of the drugs were compared with the mean frequency recorded in Ringer's solution before and after exposure to the drug. The effects of all drugs tested developed within 10 min and reversed within 30 min. Mepp frequencies were measured at least 10 min after addition of the drugs. Histograms of mepp size were obtained by plotting the mepp amplitude in 0.05–0.2 mV bins and plotting these amplitude bins against the frequency of their occurrence. Mepp size histograms obtained from one endplate in different recording solutions were compared statistically using the Kolmogorov-Smirnov test.

Adenosine, 8-p-sulfophenyltheophylline, the enzyme, adenosine deaminase, and Ro20-1724, were dissolved directly into frog Ringer's. Theophylline and 8-phenyltheophylline, at concentrations of 1–100 mM, were dissolved in 0.1 N NaOH and diluted with Ringer's to a final concentration of 1 μ M–1 mM. Drugs were added by perfusion.

Adenosine, theophylline, 8-phenyltheophylline, and adenosine deaminase (type I and X) were obtained from Sigma Chemical Co. (St. Louis), while 8-p-sulfophenyltheophylline was purchased from Research Biochemicals, Inc. (Wayland, MA). Ro20-1724 was kindly provided by Hoffmann-La Roche Inc. (Nutley, NJ).

Results

Effects of theophylline in normal Ringer's

At concentrations of 0.1 and 1.0 mM, theophylline reversibly enhanced mepp frequency in normal Ringer's solution by 45–73% (Table 1). This effect was not accompanied by a change in mepp amplitude. At concentrations of 10 and 20 μ M, theophylline produced no consistent effect on mepp frequency (Table 1). At 10 μ M, the drug did not alter the resting potential of the muscle or mepp size.

Figure 1. Stimulatory effects of 0.1 and 1.0 mM theophylline on mepp frequency in hyperosmotic Ringer's. Mepp frequencies for 30 sec recording periods were plotted against time. Theophylline enhanced mepp rate by 77% ($p < 0.001$) at 0.1 mM and by 102% ($p < 0.001$) at 1.0 mM. This effect was reversible. Inset, A recording of mepps. Theo, theophylline.

Effects of theophylline in hyperosmotic Ringer's

Since resting mepp frequency was often quite low (i.e., <10 mepps/min), mepp frequency was artificially elevated in many experiments by increasing the osmolarity of the Ringer's with the addition of 24 mM sucrose (Fatt and Katz, 1952; Furshpan, 1956; Shimoni et al., 1977). This increase in osmolarity enhanced mepp rate by 2- to 4-fold. Under these conditions, theophylline, at concentrations of 0.1 and 1.0 mM, elevated mepp frequency by 26–128%, an effect similar to its action in normal Ringer's (Fig. 1, Table 1). However, application of $10 \mu\text{M}$ theophylline produced a different effect on mepp frequency in hyperosmotic Ringer's than normal Ringer's. In hyperosmotic Ringer's, $10 \mu\text{M}$ theophylline reversibly depressed mepp frequency by an average of 34% (Fig. 2, Table 1). This action was not accompanied by any observable postsynaptic effects.

At a concentration as low as $10 \mu\text{M}$, theophylline may act as an adenosine receptor antagonist but may have little activity as an inhibitor of phosphodiesterase, 5'-nucleotidase, or alkaline phosphatase, or as a mobilizer of intracellular calcium stores. Therefore, the effect of theophylline on mepp frequency recorded in hyperosmotic Ringer's may result from adenosine receptor blockade.

Antagonism by theophylline of the effects of adenosine

Experiments were then designed to determine if micromolar concentrations of theophylline could block the effects of exogenous adenosine on neuromuscular transmission. In both normal and hyperosmotic Ringer's, addition of $10 \mu\text{M}$ adenosine to the frog NMJ reduced mepp frequency by 40–50%. A 40–50% decrease in mepp frequency is the maximum effect of adenosine on spontaneous transmitter release (Ribeiro and Sebastião, 1986). This action was partially reversed by $10 \mu\text{M}$ theophylline in 1 out of 3 experiments in which mepps were recorded in normal Ringer's and in 1 out of 2 experiments in which mepps were recorded in hyperosmotic Ringer's (Table 2). Theophylline, $10 \mu\text{M}$, also partially reversed the effects of $2 \mu\text{M}$ adenosine in one experiment in hyperosmotic Ringer's. At

$20 \mu\text{M}$, theophylline completely blocked the effects of $10 \mu\text{M}$ adenosine in 3 out of 3 experiments in which mepps were recorded in normal Ringer's (Fig. 3, Table 2). Therefore, micromolar concentrations of theophylline are capable of inhibiting the effects of maximum doses of adenosine.

Effects of other alkylxanthine drugs

The effects of theophylline were mimicked by other alkylxanthine drugs. 8-Phenyltheophylline did not alter mepp frequency in normal Ringer's (2 experiments), but in 2 out of 3 preparations, $1 \mu\text{M}$ 8-phenyltheophylline depressed mepp frequency in hyperosmotic Ringer's by 24% ($p < 0.02$) and 30% ($p < 0.001$)

Table 2. Antagonism of the depressant effect of adenosine by micromolar concentrations of theophylline

Experiment	Ringer's	Adenosine (μM)	Theophylline (μM)	% of control mepp frequency	
				Adenosine	Adenosine + theophylline
1	HR	2	10	51	66 ^a
2	HR	10	10	24	32 ^a
3	HR	10	10	53	53
4	NR	10	10	35	59 ^a
5	NR	10	10	58	67
6	NR	10	10	55	59
7	NR	10	20	58	115 ^a
8	NR	10	20	56	117 ^a
9	NR	10	20	56	98 ^a

At a concentration of $10 \mu\text{M}$, theophylline partially reversed the inhibitory effects of $10 \mu\text{M}$ adenosine on mepp frequency, while, at $20 \mu\text{M}$, theophylline completely blocked the actions of $10 \mu\text{M}$ adenosine. A 2-sample Student's *t* test was used to compare, in one preparation, mean mepp frequencies recorded in adenosine and frequencies recorded in adenosine with theophylline. NR, normal Ringer's; HR, hyperosmotic Ringer's.

^a $p \leq 0.05$.

Figure 2. Inhibitory effect of $10\ \mu\text{M}$ theophylline on mepp frequency in hyperosmotic Ringer's. **A**, mepp frequencies for 6 sec recording periods plotted against time. Theophylline reduced mepp frequency by 43% ($p < 0.01$). **B**, Amplitudes of 100 mepps recorded in the absence and 100 mepps recorded in the presence of $10\ \mu\text{M}$ theophylline were grouped into 0.05 mV size bins and plotted against the frequency of their occurrence. Theophylline did not affect the mepp amplitude distribution ($p > 0.10$). *Theo*, theophylline.

(Fig. 4). In a third experiment in hyperosmotic Ringer's, 8-phenyltheophylline did not alter mepp rate. The drug did not affect the resting potential of the muscle or mepp size. In 2 out of 2 experiments, $1\ \text{mM}$ 8-p-sulfophenyltheophylline depressed mepp frequency in hyperosmotic Ringer's by 34% ($p < 0.001$ for both preparations) but had no effect on the muscle resting potential or mepp size.

Effects of adenosine deaminase

If theophylline depresses mepp frequency by adenosine receptor blockade, then its effects should be mimicked by adenosine deaminase, an enzyme that inactivates adenosine (Daly, 1982). Application of adenosine deaminase, at doses of 2.5 units/ml, reversed the depressant effect of $10\ \mu\text{M}$ adenosine on mepp frequency. At a concentration of 0.1 unit/ml, adenosine deaminase reversibly reduced mepp frequency in hyperosmotic Ringer's by 30% ($p < 0.01$), 32% ($p < 0.02$), and 40% ($p < 0.001$) (Fig. 5). Application of 0.5 units/ml adenosine deaminase reversibly decreased spontaneous transmitter output by 20% ($p < 0.01$), 24% ($p < 0.001$), 35% ($p < 0.01$), 42% ($p < 0.001$), 65% ($p < 0.001$), and 73% ($p < 0.001$). Adenosine deaminase at a concentration of 1.0 unit/ml reduced mepp frequency by 40% ($p < 0.001$) in one preparation and enhanced mepp rate

by 21% ($p < 0.05$) in a second experiment. These actions were not accompanied by any observable postsynaptic effects. At these concentrations, adenosine deaminase did not alter mepp frequency in normal Ringer's (2 experiments, Fig. 5). Thus, the actions of adenosine deaminase paralleled the effects of theophylline on spontaneous transmitter output.

Application of adenosine deaminase to the frog NMJ masked the inhibitory effects of theophylline on mepp frequency (Fig. 6). A concentration of 0.5 units/ml of adenosine deaminase was chosen since this concentration produced a maximal inhibitory effect on spontaneous release. In 3 out of 3 experiments, mepp frequency was reduced in 0.5 units/ml adenosine deaminase. No further decrease in mepp frequency was produced by the subsequent addition of $10\ \mu\text{M}$ theophylline to the Ringer's containing adenosine deaminase. In contrast, in the same preparations, application of $10\ \mu\text{M}$ theophylline, in the absence of adenosine deaminase, depressed spontaneous release.

Effects of Ro20-1724, a phosphodiesterase inhibitor

Since theophylline, at millimolar concentrations, may act as a phosphodiesterase inhibitor, the effects of the phosphodiesterase inhibitor, Ro20-1724, were also tested on spontaneous ACh release at the frog NMJ. In normal Ringer's, Ro20-1724 re-

Figure 3. Antagonism of the inhibitory effect of adenosine on mepp frequency by theophylline. Data represent the mean $\pm$ SEM of mepp frequencies recorded during one experiment. Adenosine, at $10\ \mu\text{M}$, reduced mepp frequency by 45%, an effect completely reversed by $20\ \mu\text{M}$ theophylline. Theophylline enhanced basal mepp frequency by 25%, but this effect was not statistically significant ($p < 0.1$). *A*, adenosine; *T*, theophylline.

Figure 4. Inhibitory effect of $1\ \mu\text{M}$ 8-phenyltheophylline on mepp frequency in hyperosmotic Ringer's. Mepp frequencies for 30 sec recording periods were plotted against time. 8-Phenyltheophylline reversibly depressed mepp frequency by 30% ($p < 0.001$). *8PT*, 8-phenyltheophylline.

Figure 5. Inhibitory effect of adenosine deaminase on mepp frequency in hyperosmotic but not normal Ringer's. Mepp frequencies for 30 sec recording periods were plotted against time. Adenosine deaminase (0.1 units/ml) did not significantly affect mepp rate in normal Ringer's (13% increase, $p < 0.2$) but significantly reduced mepp frequency by 40% ($p < 0.001$) in hyperosmotic Ringer's. Data represent a continuous recording from one endplate. ADA, adenosine deaminase.

Figure 6. Presence of adenosine deaminase in the hyperosmotic Ringer's masked the depressant effect of theophylline on mepp frequency. Data represent means $\pm$ SEM of mepp frequencies recorded during one experiment. Adenosine deaminase (0.5 units/ml) reduced mepp frequency by 20% ($p < 0.01$). No further decrease in mepp frequency was produced by the subsequent addition of theophylline (10 μ M) to the hyperosmotic Ringer's containing adenosine deaminase. In the absence of adenosine deaminase, 10 μ M theophylline inhibited mepp frequency by 22% ($p < 0.01$). ADA, adenosine deaminase; THEO, theophylline.

versibly elevated mepp frequency by 49% at 60 μ M ($p < 0.05$), by 104% ($p < 0.001$) at 100 μ M, and by 465% ($p < 0.001$) at 300 μ M. This action was accompanied by a decrease in mepp size (Fig. 7). Silinsky (1984) and Silinsky and Vogel (1987) have also reported that Ro20-1724 increases mepp frequency and reduces the size of the endplate current.

Discussion

Stimulatory effects of theophylline

In normal and hyperosmotic Ringer's solution, theophylline, at concentrations at or above 100 μ M, enhanced spontaneous transmitter release from frog motoneurons. Theophylline is an adenosine receptor antagonist (Sattin and Rall, 1970; Smellie et al., 1979; Daly et al., 1981). Application of 10 μ M adenosine to the frog NMJ depressed spontaneous ACh output, an effect that was reversed by 20 μ M theophylline. Thus, theophylline may potentiate spontaneous transmitter release by blockade of adenosine receptors and inhibition of a tonic depressant effect of endogenous adenosine. Similarly, Ribeiro and Sebastião (1987) have reported that theophylline, at concentrations of 100 and 200 μ M, enhances evoked transmitter release at the frog NMJ, an effect that may result from reduction of a tonic, inhibitory action of endogenous adenosine.

At concentrations greater than or equal to 100 μ M, theophylline may also affect neuromuscular transmission by mechanisms other than adenosine receptor blockade. For example, theophylline inhibits phosphodiesterases in the rat brain with an IC_{50} of 500 μ M–1 mM (Smellie et al., 1979). The effect on transmitter release of increases in cyclic AMP levels in motor nerve terminals has not been resolved (Miyamoto and Breckenridge, 1974; Wilson, 1974; Dretchen et al., 1976; Silinsky, 1984). However, application of the phosphodiesterase inhibitor Ro20-1724 to the frog NMJ enhanced mepp frequency, an action similar to that of theophylline. Silinsky (1984) and Silinsky and Vogel (1987) have also reported that Ro20-1724 and other phosphodiesterase inhibitors increase mepp frequency. Thus, theophylline, at 100 μ M and 1 mM, may increase spontaneous transmitter release in part by phosphodiesterase inhibition and enhancement of intraterminal cyclic AMP levels.

Inhibitory effects of theophylline

In normal Ringer's, theophylline produced no consistent effect on spontaneous transmitter release at a concentration of 10 μ M. Similarly, Ribeiro and Sebastião (1985) have reported that theophylline, at concentrations at or below 50 μ M, does not affect the nerve-evoked twitch tension of frog sartorius muscle recorded in Ringer's solution of normal osmolality. In contrast, theophylline produced dual effects on spontaneous ACh output when the NMJ was bathed in hyperosmotic Ringer's. At 10 μ M, theophylline depressed mepp frequency, while at concentrations greater than or equal to 100 μ M, theophylline enhanced mepp rate. Since theophylline inhibited spontaneous transmitter release only at low micromolar concentrations, this effect may result from adenosine receptor blockade.

Other alkylxanthine drugs, such as 8-phenyltheophylline and 8-p-sulfophenyltheophylline, also depressed mepp frequency when the frog NMJ was exposed to hyperosmotic Ringer's. 8-Phenyltheophylline is a more potent adenosine receptor antagonist than theophylline but has little activity as a phosphodiesterase inhibitor (Smellie et al., 1979). 8-p-Sulfophenyltheophylline crosses cell membranes only to a limited extent, so that its effects may be confined to adenosine receptor blockade (Daly, 1982).

The effects of theophylline were also mimicked by adenosine deaminase, an enzyme that converts adenosine to inosine, a metabolite with no effect on neuromuscular transmission (Ginsborg et al., 1973; Ribeiro and Sebastião, 1987). In addition, the inhibitory effects of theophylline (10 μ M) were not seen when adenosine deaminase (0.5 units/ml) was already present in the hyperosmotic Ringer's. Thus, theophylline and adenosine deaminase may both reduce spontaneous transmitter release from frog motoneurons because they both inhibit the effects of endogenous adenosine. These results indicate that endogenous adenosine may have a second, stimulatory effect on spontaneous transmitter release.

The inhibitory and stimulatory actions of adenosine on spontaneous ACh release may be mediated by different types of

Figure 7. Stimulatory effect of Ro20-1724 on mepp frequency. **A**, Mepp frequencies for 1 min recording periods were plotted against time. Ro20-1724, at 300 μ M, reversibly enhanced mepp rate by 465% ($p < 0.001$). **B**, This effect was accompanied by a decrease in mepp size ($p < 0.001$). Mepp amplitudes for 100 mepps in the absence and 100 mepps in the presence of 300 μ M Ro20-1724 were grouped into 0.05 mV size bins and plotted against the frequency of their occurrence. Recordings were made in normal Ringer's.

adenosine receptors on motor nerve terminals. Several classes of adenosine receptors have been found on the external membranes of many cell types, including neurons (Londos and Wolff, 1977; Van Calker et al., 1978, 1979; Daly et al., 1981). At A_1 receptors, adenosine inhibits while at A_2 receptors, adenosine stimulates the activity of the enzyme adenylyl cyclase. In addition, a third class of adenosine receptors, A_3 , which may be coupled to calcium channels, may be found on nerve endings (Ribeiro and Sebastião, 1986). These receptors can be distinguished pharmacologically and are all blocked by theophylline and other alkylxanthine drugs.

When adenosine is applied to the frog NMJ, its most common effect is to depress mepp frequency (Ribeiro and Walker, 1975; Brânisteanu et al., 1979; Silinsky, 1984). Ribeiro and Sebastião (1986) have suggested that the inhibitory effect of adenosine on spontaneous release results from an intracellular action of adenosine. However, the depressant effect of adenosine on mepp frequency is blocked by theophylline (Fig. 3). Furthermore, several impermeable adenosine analogs such as L- and D-phenylisopropyladenosine (PIA) and 5'-N-ethylcarboxamidoadenosine (NECA) also depress mepp frequency (see Barry, 1987). L-PIA is 15 times more potent than NECA and 200 times more effective than D-PIA in reducing spontaneous ACh release (Barry, 1987). The rank-order potency of these analogs in depressing spontaneous release differs from their effectiveness in inhibiting evoked release (Ribeiro and Sebastião, 1985, 1986) and suggests that adenosine inhibits spontaneous, but not evoked, release by acting at an A_1 -like receptor.

High concentrations of adenosine have also been reported to enhance spontaneous transmitter release (Silinsky, 1980). Furthermore, NECA, a mixed A_1 - A_2 adenosine agonist, produces dual effects on mepp frequency (Barry, 1986b). Thus, adenosine may stimulate as well as inhibit spontaneous transmitter release. This effect may be mediated by a second adenosine receptor, perhaps of the A_2 subtype.

In normal Ringer's, the primary effect of endogenous adenosine may be to depress spontaneous ACh release. Thus, theophylline has only one action in normal Ringer's, to enhance spontaneous release. In hyperosmotic Ringer's, endogenous adenosine may both stimulate and depress spontaneous transmitter output. Thus, theophylline, in hyperosmotic Ringer's, may decrease spontaneous release at low concentrations and increase spontaneous output at high concentrations. The stimulatory effects of adenosine may be blocked at lower concentrations of theophylline than the inhibitory effects.

The effects of endogenous adenosine may differ in normal and hyperosmotic Ringer's because the source of adenosine produced endogenously at the frog NMJ may vary with the osmolarity of the Ringer's. Adenosine uptake systems, which are present on nerve terminals and on glial cells, and extracellular hydrolyzing enzymes, such as adenosine deaminase, may confine adenosine's actions to receptors located near the source of adenosine (Dowdall, 1978; Daly, 1982; Ribeiro and Sebastião, 1987). Adenosine receptors which mediate a decrease in spontaneous transmitter release may be accessible to exogenously applied adenosine or to adenosine produced endogenously in normal Ringer's. In contrast, the receptors that mediate an increase in spontaneous output may be accessible to adenosine that is produced endogenously in hyperosmotic Ringer's.

Endogenous adenosine at the NMJ may originate from several sources. Adenosine triphosphate (ATP) is released with ACh from motor nerve terminals (Silinsky and Hubbard, 1973; Silinsky, 1975) and may be hydrolyzed to adenosine by ectoenzymes in the synaptic cleft (Dowdall, 1978; Fredholm and Hedqvist, 1980; Ribeiro and Sebastião, 1987). Extracellular adenosine may be produced by this mechanism in both normal and hyperosmotic Ringer's. In addition, cells that are metabolically very active secrete adenosine which is probably produced from the intracellular hydrolysis of ATP and cyclic AMP (Fredholm and Hedqvist, 1980; Stone, 1981). When the frog NMJ is placed in hyperosmotic Ringer's solution, extracellular adenosine may be generated by such a process. For example, the increase in mepp frequency observed in hyperosmotic Ringer's may result from an increase in intracellular calcium concentration within the motor nerve terminal (Shimoni et al., 1977). This elevation in calcium levels may activate intraterminal ATP-dependent calcium sequestration systems (Blaustein et al., 1978), causing increased hydrolysis of ATP and enhanced secretion of adenosine. The adenosine, thus produced, may be accessible to stimulatory but not inhibitory receptors on the nerve terminal and may tonically enhance spontaneous transmitter release. It is this second, stimulatory effect of adenosine that may be inhibited by application of 10 μ M theophylline, other alkylxanthines, and adenosine deaminase.

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