Br. J. Pharmacol. (1988), **93**, 839–845

The effect of reduced temperature on the inhibitory action of adenosine and magnesium ion at frog motor nerve terminals

[1]Eugene M. Silinsky & Jody K. Hirsh

Department of Pharmacology, Northwestern University Medical School, 303 East Chicago Avenue, Chicago, Illinois 60611, U.S.A.

1 A study was made to exclude the notion that adenosine receptor agonists exert a direct physical blockade of the depolarization-secretion process. Reduced temperature was employed as a tool for distinguishing between physico-chemical processes (such as those which mediate evoked transmitter release) and biochemical mechanisms (such as those which involve second messenger substances) in the action of adenosine. Adenosine and 2-chloroadenosine were used as agonists in this electrophysiological study of the release of acetylcholine (ACh) from frog motor nerve terminals.

2 The ability of these two adenosine receptor activators to reduce neurally-evoked ACh release was prevented or greatly attenuated by maintaining the preparation at temperatures between 5 and 10°C. Such low temperatures inhibit the activation of receptors coupled to second messengers via guanine nucleotide binding proteins (e.g. adenylate cyclase). Low temperature alone did not substantially alter evoked ACh secretion under the conditions of these experiments.

3 Inhibition of evoked ACh release by the extracellular Ca antagonist Mg, which acts directly to block Ca channels, was not affected by low temperature.

4 The results are consistent with the hypothesis that a temperature-sensitive second messenger system controls the intracellular events linked to extracellular adenosine receptor activation.

Introduction

Adenosine is a ubiquitous neuromodulatory substance which may play a role in the presynaptic inhibition of acetylcholine (ACh) release (Silinsky, 1975; Ribeiro, 1979; Sebastiao & Ribeiro, 1985; for general reviews see Stone, 1981; Phillis & Wu, 1982; Dunwiddie, 1985). At the motor nerve ending, adenosine receptor activation is likely to depress quantal ACh release by causing a decrease in the apparent affinity for Ca of a structural component of the secretory apparatus (Silinsky, 1981; 1984; but see Ribeiro & Sebastiao, 1986).

As the adenosine receptor responsible for inhibition of ACh release is situated extracellularly (Silinsky, 1980; Daly, 1985; Sebastiao & Ribeiro, 1985), adenosine may be exerting its inhibitory effect directly by a physico-chemical linkage to cellular Ca binding proteins or indirectly via the action of a secondary or tertiary messenger, e.g. guanine nucleotide binding (G) proteins, cyclic adenosine monophosphate (cyclic AMP) (see Silinsky, 1986). It should be possible to distinguish between these two broad mechanisms by relying on the more pronounced effects of temperature on enzyme-catalysed reactions as compared to physico-chemical processes such as ion diffusion and binding (see Hille, 1984, pp. 198–202). For example, hormonal stimulation of adenylate cyclase by its G protein (Gs) is severely depressed at temperatures below room temperature (Harwood & Rodbell, 1973; Codina *et al.*, 1984) with complete inhibition occurring near 4°C (Codina *et al.*, 1984). In contrast, a predominantly physico-chemical process such as the evoked release of acetylcholine (Parsegian, 1977) may not be greatly altered in the temperature range expected to inhibit the G protein/adenylate cyclase system (see e.g. Jensen, 1972; Barrett *et al.*, 1978).

Based upon the above considerations, if adenosine continues to exert its usual inhibitory effects on motor nerve endings at low temperature, then it is unlikely that G proteins or cyclic AMP are mediating the action of adenosine. If, however, the effects of adenosine are inhibited at the low temperatures,

[1]Author for correspondence.

then this would argue against a direct physical coupling between Ca binding proteins and adenosine receptors (for a depiction of this coupling see Silinsky, 1986; Figure 3) and in favour of mediation through second messengers.

Methods

Electrophysiological procedures

Cutaneous pectoris nerve-muscle preparations of frog (*Rana pipiens*) were dissected and superfused with flowing Ringer solution. For experiments in which the temperature was altered, the superfusion solution was encircled by a Perspex column, which in turn was connected to a Lauda (Brinkmann) circulator. The bath temperature was measured with a Yellow Springs Instruments thermistor with a microprobe positioned in the bathing solution near the recording electrode. Supramaximal stimulation pulses were delivered to the nerve supply through a suction electrode. Intracellular recordings were made at endplate regions by use of glass microelectrodes filled with 3 M KCl and with resistances from 8–25 MΩ. Signals from the microelectrodes were fed into a conventional high input impedance preamplifier, the output of which was delivered into an oscilloscope, a computer, a pen recorder (Brush-Gould), and an FM tape recorder.

In some experiments, endplate potential (e.p.p.) amplitudes were averaged by a computer of average transients (Nicolet) in which case the average was displayed on the pen recorder. In the majority of instances, experiments were controlled and analysed with a LSI 11/73 computer (Cambridge Digital) and 125 kHz 14 bit A/D-12 bit D/A converter on line with hard copy of the digitalized traces being made on an XY plotter (Hewlett Packard 7470). Miniature endplate potential (m.e.p.p.) frequencies were determined from pen records and m.e.p.p. amplitudes from pen records after first being recorded on magnetic tape.

Measurements of quantal ACh release

In the absence of tubocurarine (Tc), the mean number of ACh quanta released synchronously by a nerve impulse ($\bar{m}$), was determined from the ratio of the mean computer averaged e.p.p. to the mean m.e.p.p. amplitude (del Castillo & Katz, 1954) using the formamide method to block muscle contraction (see del Castillo & Motta, 1978; Silinsky & Vogel, 1986). In the experiments in Tc (1.5–8 mg l^{-1}), changes in e.p.p. amplitudes, which accurately reflect changes in synchronous ACh release in adenosine (Ginsborg & Hirst, 1972; Silinsky, 1980; 1981; 1984), were used as a measure of presynaptic effects of adenosine and 2-chloroadenosine. For the temperature studies, only evoked release reflected as e.p.ps was quantitated as m.e.p.p. frequencies are reduced to such low levels below 8°C as to render frequency measurements technically impractical (see Results).

The statistical procedures were identical to those described previously (see Silinsky, 1984, pp. 244–245). In most instances, appropriate averaging techniques were employed (see Silinsky, 1984; 1987). Statistically significant differences were generally observed at $P \ll 0.01$. In instances where significance was at the $P < 0.05$ level, this is stated in the text (see e.g. legend to Figure 3). Corrections for non-linear summation were employed when necessary (McLachlan & Martin, 1981).

Composition of solutions and chemicals

Normal frog Ringer contained (mM): NaCl 115, KCl 2, $CaCl_2$ 1.8, $NaHCO_3$ 2 (pH 7.2–7.4) and was used in all experiments. Drugs were obtained from the Sigma Chemical Company.

Results

The effect of temperature on quantal ACh release in normal calcium solutions

As a prelude to studies with adenosine derivatives, it is necessary to confirm directly that synchronous evoked ACh release, a process unlikely to involve extensive enzyme catalysis (Parsegian, 1977; Silinsky, 1985b) remains near normal at the low temperatures which inhibit G protein-linked second messenger systems (Harwood & Rodbell, 1973; Codina *et al.*, 1984). Figure 1, which illustrates an experiment made in normal Ca solution after formamide treatment, provides support for this contention. In Figure 1, whilst the predicted changes in the kinetics of the e.p.ps (a) and the m.e.p.ps (b and c) are evident as the temperature is varied between 8 and 20°C (del Castillo & Machne, 1953; Li & Gouras, 1958; Katz & Miledi, 1965; Jensen, 1972; Barrett *et al.*, 1978), the ratio of the mean e.p.p. to the mean m.e.p.p. amplitude ($\bar{m}$) remained near the control level ($\bar{m} = 14$ at 8°C, $\bar{m} = 17$ at 20°C; see also Jensen (1972), Figure 2 and Table 1).

In contrast to the results on evoked release, the rate of spontaneous ACh release was markedly reduced by lowering the temperature (see Figure 1, legend), confirming observations made by Barrett *et al.* (1978) in the frog cutaneous pectoris nerve-muscle preparation.

As the evoked ACh release mechanism functions at near normal capacity at low temperature, it would

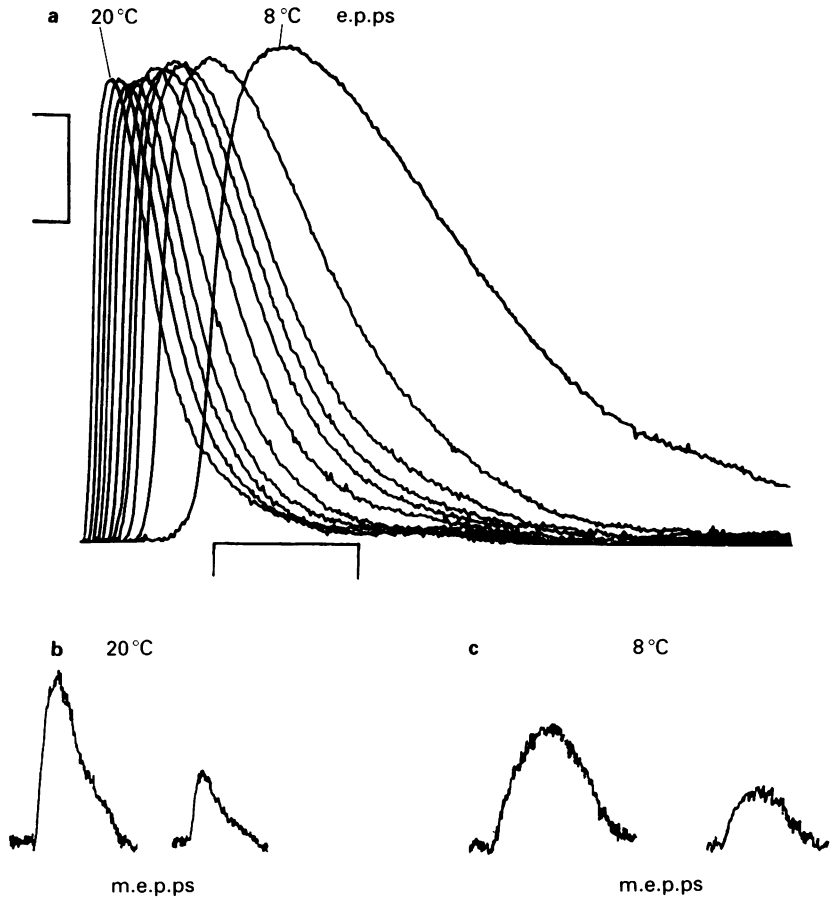

Figure 1 Temperatures between 8–20°C produce minimal effects on evoked acetylcholine (ACh) release ($\bar{m}$) in normal Ca solutions. The $\bar{m}$ was measured directly from the ratio of the mean endplate potential amplitude (e.p.p., (a)) to the miniature endplate potential amplitudes (m.e.p.p., (b) and (c)) using the formamide method to block excitation-contraction coupling. The $\bar{m}$ at 20°C was 17.4 whilst $\bar{m}$ at 8°C was 14.1. (a) Shows progressive slowing of e.p.p. kinetics and lengthening of latency period without substantial changes in amplitude as temperature is lowered progressively from 20°C (left trace), from 17–9°C (middle 9 traces) and 8°C (right trace). Each trace is the average of 4 e.p.ps (stimulation frequency 0.1 Hz). (b) Shows two representative m.e.p.ps at 20°C (c) shows two m.e.p.ps at 8°C. Note the slower kinetics of the low temperature m.e.p.ps. E.p.ps and m.e.p.ps were corrected from the raw data shown in the figure for small changes in resting potential. Vertical calibrations 5 mV for (a), 2 mV for (b) and (c). Horizontal calibration, 10 ms. The m.e.p.p. frequency (per s) was decreased from the control value at 20°C of 0.87 ± 0.21 (mean $\pm$ s.e. mean, $n = 38$) to 0.21 ± 0.04 ($n = 81$) at 8°C.

be of interest to compare the effects of adenosine derivatives at normal temperature and at low temperatures expected to inhibit receptor systems linked through G proteins.

Inhibitory effects of low temperature on the action of adenosine receptor agonists

At room temperature (20°C) adenosine and 2-chloroadenosine have both been found to produce a maximal inhibitory effect on evoked ACh release of approximately 50% of the control value (Ginsborg & Hirst, 1972; Silinsky, 1980; 1984). The latter compound is from 1–2 orders of magnitude more potent than the parent nucleoside with maximal inhibition of $\bar{m}$ by 2-chloroadenosine generally observed at 500 nM (see Silinsky, 1984, Figure 1). At 5°C, however, in the experiment illustrated in Figure 2, 500 nM 2-chloroadenosine was completely devoid of inhibitory effects on evoked ACh release. The absence of a statistically-significant inhibitory effect of 2-chloroadenosine (500 nM–10 μM) or adenosine

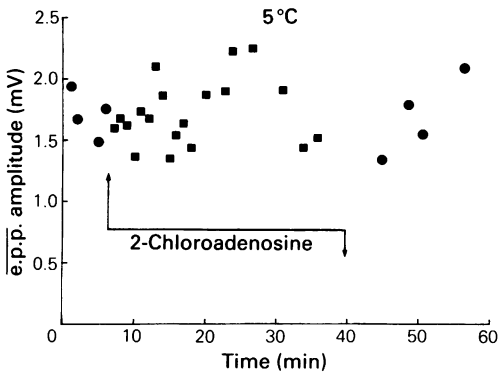

Figure 2 Absence of effects of 2-chloroadenosine (500 nM) on evoked acetylcholine (ACh) release in normal Ca solutions at 5°C: (■) e.p.ps in 2-chloroadenosine (applied between arrows). Changes in e.p.p. amplitudes in the presence of adenosine derivatives accurately reflect changes in ACh release (e.g. Ginsborg & Hirst, 1972). Normal Ca Ringer contained tubocurarine 2 mg l^{-1}. Control e.p.ps = (●) (averaged response to 4 stimuli at 0.1 Hz).

(50 μM) at low temperature (5–10°C) was reproduced in 5 of 6 experiments, with a modest inhibitory effect being observed in the sixth (see Figure 3b below).

It would be of interest to determine what contribution changes in agonist binding make to the paucity of effects of adenosine receptor agonists at low temperature. The fortuitous discovery of a nerve ending in which adenosine is a particularly efficacious inhibitor of ACh release at normal temperature (Figure 3a, 26% of control) made such an investigation possible. Figure 3 illustrates the concentration-effect relationship for adenosine at two different temperatures in the same fibre. At low temperature (6°C, Figure 3b), despite the small maximal inhibitory effect (only to 77% of control, open and closed squares), the relationship between adenosine concentration and percentage maximal inhibition is similar to that published previously (see Figure legend and Silinsky, 1984; Figure 1). The observation that there was little or no change in the concentration of adenosine required for half-maximal inhibition at 6°C as at 20°C (see Figure legend) suggests that differences in binding at normal and low temperatures are not responsible for the failure of adenosine derivatives to inhibit ACh release at reduced temperatures. Rather, it appears that low temperature reduces the maximal inhibitory efficacy of adenosine receptor agonists.

Effects of low temperature on the action of Mg

Mg has been found to block evoked ACh release by occluding the site of Ca entry in a competitive manner at motor nerve endings (Jenkinson, 1957; Kharasch *et al.*, 1981). If reduced temperature were selectively impairing the effects of agents that act through second messengers, then, blockade of release by Mg would not be affected by low temperature as antagonism by the cation is by a direct interaction with the ion channels. Figure 4 shows this to be the case. Specifically, in contrast to the impaired action of adenosine at low temperature, inhibition by 5 mM Mg at low temperature was in the range found previously at normal temperature in frog preparations. This is also the level of inhibition expected from simple equilibrium considerations (Figure 4; cf. Silinsky, 1984, Methods and Figures 3 and 7; Jenkinson, 1957)

Discussion

These results reinforce the notion that secondary and or tertiary messenger substances such as those linked to G-proteins are involved in the inhibition of ACh secretion by adenosine derivatives. At the reduced temperature (5–10°C) expected to have only minor effects on physico-chemical processes (Hille, 1984) yet able to inhibit the enzyme adenylate cyclase and other second messenger systems linked to G proteins (Harwood & Rodbell, 1973; Codina *et al.*, 1984; Hille, 1984), the inhibitory effect of adenosine derivatives is prevented or severely impaired. The observation that Mg still retains its typical inhibitory effects at low temperature provides further evidence for the temperature-independence expected of direct acting Ca antagonists. It also appears that a physico-chemical antagonism of the Ca channel in a manner equivalent to Mg is not responsible for the blockade of evoked release by adenosine.

It is unclear as to what specific component of the action of adenosine nucleosides is being affected by lowered temperature. The results of Figure 3b suggest that it is not the initial adsorption of adenosine onto its extracellular binding site that is impaired; rather, it is the effectiveness by which receptor-bound adenosine elicits its response that is decreased by a reduction in the temperature of the bathing fluid. It is also unlikely that low temperature is inhibiting ACh release by reducing the uptake of adenosine to a target site within the nerve ending. Firstly, an extracellular receptor of the A/R category (Daly, 1985) is responsible for the inhibitory effects of adenosine at motor nerve endings (Silinsky, 1980; Ribeiro & Sebastiao, 1985) and a reduction in uptake would be expected to increase the effectiveness of a submaximal concentration of adenosine, not impair it. In addition, 2-chloroadenosine is a minimal substrate for uptake and deamination in

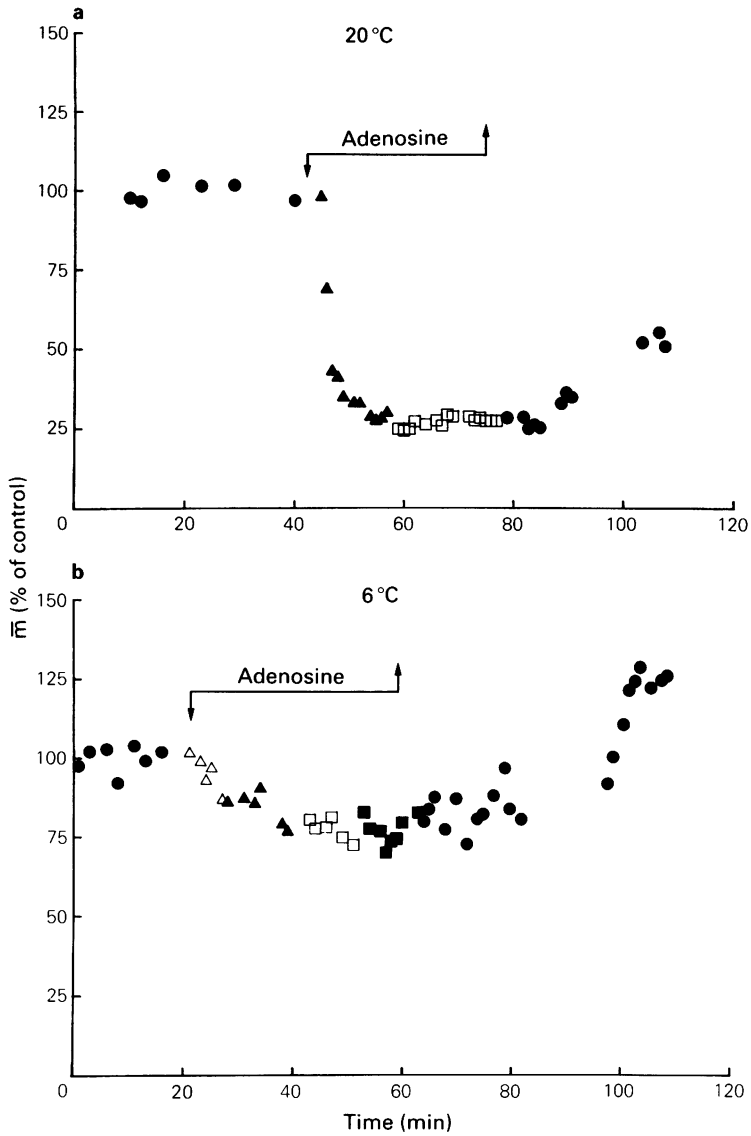

Figure 3 Concentration-effect relationship for adenosine in normal calcium solutions at room temperature (20°C; a) and at low temperature (6°C; b) in the same fibre. Each symbol is the averaged response to 4 stimuli delivered at 0.1 Hz. (a) Shows that, in this cell, adenosine was a particularly efficacious inhibitor of ACh release at room temperature; a reduction to approximately 26% of the control value was observed rather than the usual 50% inhibition. Adenosine concentrations were 10 μM (▲) and 25 μM (□); significant differences in inhibition were observed between the two concentrations ($P < 0.05$). Maximal inhibition is usually observed at a concentration of 25 μM adenosine (see Silinsky, 1980; 1984). The tubocurarine (Tc) concentration was 4 mg l^{-1}. In (b) made at 6°C, 1 μM adenosine produced a statistically-significant decrease ($P < 0.05$) in ACh release (△) relative to control (●) with a further statistically-significant effect being observed with 10 μM adenosine (▲). The maximal inhibitory effect (approximately 77% of control) was achieved at 25 μM; i.e., no significant differences were observed between 25 and 50 μM adenosine (■). The issue of whether 10 μM adenosine is producing a maximal response is uncertain given the small effects seen at low temperature. For example if the last three averaged responses in 10 μM adenosine are compared to the last three averages in 25 μM adenosine (□), then the differences are statistically significant. If however 10 μM is compared to all of the averaged data points in 50 μM adenosine, then no significant differences arise. The average points in 50 μM adenosine, however, may show additional scatter because of the tendency of high concentrations of adenosine receptor agonists to produce rebound increases in ACh secretion. These increases may begin during exposure (Silinsky, 1980) but are most pronounced after exposure to agonist (this is seen at the end of Figure 3b, (●)). It is apparent, however, that low temperature does not reduce the potency of adenosine. Normal Ca Ringer contained Tc (1 mg l^{-1}). See text for interpretation of the results.

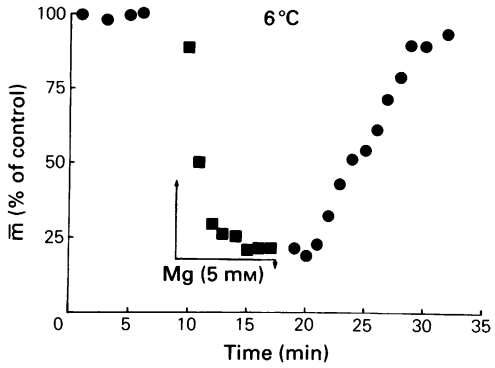

Figure 4 Inhibitory effect of Mg (5 mM) on evoked acetylcholine release at 6°C. This inhibitory effect is within the range of that observed at room temperature (see Silinsky, 1984; Figure 3). Control = (●); Mg = (■) (between arrows). Data are from the same experiment as Figure 3.

neural elements (Daly, 1982) yet its effects are impaired at low temperature.

It may be speculated that a G protein linked to the adenosine receptor either directly or through a second messenger (e.g. cyclic AMP) is prevented from attaining its activated state. Indeed, it has been shown that the lowest temperatures employed in this study (5°C) prevent the dissociation of the alpha subunit from the regulatory guanine nucleotide binding complex of the G protein; dissociation of the alpha subunit from the alpha/beta/gamma complex is essential for the binding of agonists to be coupled to biological effects (Codina *et al.*, 1984). G-proteins have been implicated as mediators of the action of adenosine (Dolphin & Prestwich, 1985; Kurachi *et al.*, 1986; Silinsky, 1985a) although the issues of guanine nucleotide binding proteins and cyclic AMP are still unclear at motor nerve endings (Silinsky *et al.*, 1987). Experiments are currently underway using lipid vesicles to deliver various G-protein and cyclic nucleotide reagents to the nerve terminal cytoplasm to test whether G-proteins directly or indirectly (through changes in cyclic AMP concentrations) affect the action of adenosine.

The classification of adenosine receptor on prejunctional nerve endings is controversial at present. Some recent evidence including experiments whereby cyclic AMP liposomes mimic the effects of adenosine (Silinsky *et al.*, 1987, Figure 6) suggest that an A_2 receptor (associated with increases in cyclic AMP) might be involved in the presynaptic effects of adenosine. (See also Discussion in Silinsky & Vogel, 1986 and Phillis & Barraco, 1985 for additional evidence of prejunctional A_2 sites which inhibit transmitter release). However, A_1 receptors (which produce decreases in cyclic AMP) or perhaps even further divisions such as an A_1 subtype (see Dunwiddie & Proctor, 1987) or an A_3 receptor (coupled to Ca binding proteins or to Ca entry – see Ribeiro & Sebastiao, 1986) may need to be postulated to explain the prejunctional affects of adenosine and some synapses.

In conclusion, regardless of the nature of the biochemical messenger substance or the subtype of extracellular adenosine receptor, the results described herein appear to exclude a direct physicochemical process such as that which evokes ACh release in mediating the inhibitory effects of the action of adenosine.

We acknowledge the kindness of the Neurological Sciences Study Section of the National Institutes of Health for U.S. Public Health Service Grant NS 12782 which supported this work. J.H. was supported by a predoctoral fellowship from the Lucille P. Markey Foundation.

References

BARRETT, E.F., BARRETT, J.N., BOTZ, D., CHANG, D.B. & MAHAFFEY, D. (1978). Temperature-sensitive aspects of evoked and spontaneous transmitter release at the frog neuromuscular junction. *J. Physiol.*, **279**, 253–273.

CODINA, J., HILDEBRANDT, J.D., BIRNBAUMER, L. & SEKURE, R.D. (1984). Effects of guanine nucleotides and Mg on human erythrocyte Ni and Ns, the regulatory component of adenylate cyclase. *J. Biol. Chem.*, **259**, 11408–11418.

DALY, J.W. (1982). Adenosine receptors: targets for future drugs. *J. Med. Chem.*, **25**, 197–207.

DALY, J.W. (1985). Adenosine receptors. In *Advances in Cyclic Nucleotide and Protein Phosphorylation Research*, Vol. 19. ed. Cooper, D.M.F. & Seamon, K.B. pp. 29–46. New York: Raven Press.

DEL CASTILLO, J. & DE MOTTA, G.E. (1978). A new method for excitation contraction uncoupling in frog skeletal muscle. *J. Cell Biol.*, **78**, 782–784.

DEL CASTILLO, J. & KATZ, B. (1954). Quantal components of the end-plate potential. *J. Physiol.*, **124**, 560–573.

DEL CASTILLO, J. & MACHNE, X. (1953). Effect of temperature on the passive electrical properties of the muscle fibre membrane. *J. Physiol.*, **120**, 431–434.

DOLPHIN, A.C. & PRESTWICH, S.A. (1985). Pertussis toxin reverses adenosine inhibition of neuronal glutamate release. *Nature*, **316**, 148–150.

DUNWIDDIE, T.V. (1985). The physiological role of adenosine in the central nervous system. *Int. Rev. Neurobiol.*, **27**, 63–139.

DUNWIDDIE, T.V. & PROCTOR, W.R. (1987). Mechanisms underlying physiological responses to adenosine in the central nervous system. In *Topics and Perspectives in*

Adenosine Research. ed Gerlach, E. & Becker, B.F. pp. 499–508. Berlin Heidelberg: Springer-Verlag.

GINSBORG, B.L. & HIRST, G.D.S. (1972). The effect of adenosine on the release of the transmitter from the phrenic nerve of the rat. *J. Physiol.*, **224**, 629–645.

HARWOOD, J.M. & RODBELL, M. (1973). Inhibition by fluoride ion of hormonal activation of fat cell adenylate cyclase. *J. Biol. Chem.*, **248**, 4901–4904.

HILLE, B. (1984). *Ionic Channels of Excitable Membranes*, Sunderland, MA., U.S.A.: Sinauer Assoc. Inc.

JENKINSON, D.G. (1957). The nature of the antagonism between calcium and magnesium ions at the neuromuscular junction. *J. Physiol.*, **138**, 434–444.

JENSEN, D.W. (1972). The effect of temperature on transmission at the neuromuscular junction of the sartorius muscle of *Rana pipiens*. *Comp. Biochem. Physiol.*, **43A**, 685–695.

KATZ, B. & MILEDI, R. (1965). The effect of temperature on the synaptic delay at the neuromuscular junction. *J. Physiol.*, **181**, 656–670.

KHARASCH, E.D., MELLOW, A.M. & SILINSKY, E.M. (1981). Intracellular magnesium does not antagonize calcium dependent acetylcholine secretion. *J. Physiol.*, **314**, 255–263.

KURACHI, Y., NAKAJIMA, T. & SUGIMOTO, T. (1986). On the mechanism of activation of muscarinic K channels by adenosine in isolated atrial cells: involvement of GTP-binding proteins. *Pflügers Archiv.*, **407**, 264–274.

LI, C-L. & GOURAS, P. (1958). Effect of cooling on neuromuscular transmission in the frog. *Am. J. Physiol.*, **192**, 464–474.

McLACHLAN, E.M. & MARTIN, A.R. (1981). Non-linear summation of end-plate potentials in the frog and mouse. *J. Physiol.*, **311**, 307–324.

PARSEGIAN, V.A. (1977). Considerations in determining the mode of influence of calcium on vesicle-membrane interactions. In *Approaches to the Cell Biology of the Neuron.* ed. Cowan, W.M. & Ferrendelli, J.A., pp. 161–171. Bethesda: Society for Neurosciences Symposia.

PHILLIS, J.W. & BARRACO, R.A. (1985). Adenosine, adenylate cyclase, and transmitter release. In *Advances in Cyclic Nucleotide and Protein Phosphorylation Research*, Volume 19, ed. Cooper, D.M.F. & Seamon, K.B., pp. 243–257. New York: Raven Press.

PHILLIS, J.W. & WU, P. (1982). Adenosine and adenosine triphosphate as neurotransmitter/neuromodulator in the brain: The evidence is mounting. In *Trends in Autonomic Pharmacology*, Volume 2. ed. Kalsner, S. pp. 237–261. Baltimore: Urban & Schwartzenberg.

RIBEIRO, J.A. (1979). Purinergic modulation of transmitter release. *J. Theor. Biol.*, **80**, 259–270.

RIBEIRO, J.A. & SEBASTIAO, A.M. (1985). On the type of receptor involved in the inhibitory action of adenosine at the neuromuscular junction. *Br. J. Pharmacol.*, **84**, 911–918.

RIBEIRO, J.A. & SEBASTIAO, A.M. (1986). Adenosine receptors and calcium: basis for proposing a third (A3) adenosine receptor. *Prog. Neurobiol.*, **26**, 279–309.

SEBASTIAO, A.M. & RIBEIRO, J.A. (1985). Enhancement of transmission at the frog neuromuscular junction by adenosine deaminase, evidence for an inhibitory role of endogenous adenosine on neuromuscular junctions. *Neurosci. Letters*, **62**, 267–270.

SILINSKY, E.M. (1975). On the association between transmitter secretion and the release of adenine nucleotides from mammalian motor nerve terminals. *J. Physiol.*, **247**, 145–162.

SILINSKY, E.M. (1980). Evidence for specific adenosine receptors at cholinergic nerve endings. *Br. J. Pharmacol.*, **71**, 191–194.

SILINSKY, E.M. (1981). On the calcium receptor that mediates depolarization-secretion coupling at cholinergic motor nerve terminals. *Br. J. Pharmacol.*, **73**, 413–429.

SILINSKY, E.M. (1984). On the mechanism by which adenosine receptor activation inhibits the release of acetylcholine from motor nerve endings. *J. Physiol.*, **346**, 243–256.

SILINSKY, E.M. (1985a). Calcium and transmitter release: modulation by adenosine derivatives. In *Calcium in Biological Systems*, ed. Rubin, R.P., Weiss, G.B. & Putney, J.W. Jr., pp. 109–119. New York: Plenum Press.

SILINSKY, E.M. (1985b). The biophysical pharmacology of calcium-dependent acetylcholine secretion. *Pharmacol. Rev.*, **37**, 81–132.

SILINSKY, E.M. (1986). Inhibition of transmitter release by adenosine: Are calcium currents depressed or are the intracellular effect of calcium impaired? *Trends Pharmac. Sci.*, **7**, 180–195.

SILINSKY, E.M. (1987). Electrophysiological methods for studying acetylcholine secretion. In *The Secretory Process* Volume 3, *In Vitro* Methods For Studying Secretion. ed: Poisner, A.M. & Trifaro, J.T., pp. 255–271. Amsterdam: Elsevier.

SILINSKY, E.M. & VOGEL, S.M. (1986). The effects of an adenylate cyclase inhibitor on the electrophysiological correlates of neuromuscular transmission in the frog. *Br. J. Pharmacol.*, **88**, 799–805.

SILINSKY, E.M., HIRSH, J.K. & VOGEL, S.M. (1987). Intracellular calcium mediating the actions of adenosine at neuromuscular junctions. In *Topics and Perspectives in Adenosine Research.* ed. Gerlach, E. & Becker, B.F. pp. 537–548. Berlin, Heidelberg: Springer-Verlag.

STONE, T.W. (1981). Physiological roles for adenosine and adenosine 5′-triphosphate in the nervous system. *Neurosci.*, **6**, 523–555.

(Received June 22, 1987
Revised October 5, 1987
Accepted November 6, 1987)