The Actions of Adenosine and Some Analogues on Evoked and Potassium Stimulated Release at Skeletal and Autonomic Neuromuscular Junctions

P. J. Buckle and I. Spence
Roche Research Institute of Marine Pharmacology, PO Box 299, Dee Why, NSW 2099, Australia

Summary. The actions of the nucleosides adenosine, 1-methyladenosine, 1-methylisoguanosine and 2-chloroadenosine on transmitter release at the mammalian neuromuscular junction and the vas deferens have been examined. All the nucleosides depressed the evoked release of acetylcholine at the neuromuscular junction, the order of potency being 2-chloroadenosine > 1-methylisoguanosine > 1-methyladenosine > adenosine. This correlated reasonably with the potency of these compounds in depressing spinal reflexes in anaesthetized mice (Buckle and Spence 1981). Neither adenosine (0.5 and 1.0 mmol 1⁻¹) or 1-methylisoguanosine (10 and 20 μmol 1⁻¹) had any effect on the elevation of miniature end-plate potential frequency caused by 120 mmol 1⁻¹ K⁺ at the neuromuscular junction. In the guinea-pig vas deferens, however, 1-methylisoguanosine and adenosine were approximately equipotent in depressing overflow of radioactively labelled noradrenaline. The actions of the nucleosides have been compared with their effects on adenylate cyclase and their ability to resist uptake and deamination. It is concluded that the relative potencies of the nucleosides are not determined solely by their ability to survive in the extracellular fluid.

Key words: Nucleosides analogues and derivatives - Neuromuscular junction - Vas deferens

Introduction

In a previous paper (Buckle and Spence 1981) the actions of a series of analogues of adenosine on spinal reflexes and neuromuscular transmission in vivo were described. One conclusion of this study was that all the nucleosides appeared to act at a common site and that their differences in potency related to the ability of some of the compounds to resist degradation. We have extended this study in the present work to examine the effects of some of these adenosine analogues on neuromuscular transmission at the skeletal neuromuscular junction and the vas deferens. As adenosine itself has been shown to block the evoked release of both acetylcholine (Ginsborg and Hirst 1972) and noradrenaline (Farnebo and Malmfors 1971) at these junctions, it seemed desirable to establish whether or not these analogues also have this effect. A second aim of the study was to examine the effects of purine nucleosides on potassium stimulated release of transmitter, in the hope that this might shed some light on the mechanism of action of these compounds in depressing transmitter release.

Methods

Biological Preparations and Recording Methods

Vas Deferens. Albino male guinea-pigs (Füllinsdorf strain 350 - 500 g) were stunned and bled. The vasa deferentia were dissected free of the mesentery and removed. A 15 mm length of each vas was then removed from the prostatic end and incubated for 30 min at 37°C in modified Krebs' bicarbonate solution containing (mmol 1⁻¹): NaCl 133; KCl 4.7; CaCl₂ 2.5; NaH₂PO₄ 1.3; MgSO₄ 1.2; NaHCO₃ 16; glucose 8; sodium ascorbate 0.6 and 0.1 μmol 1⁻¹ ³H-noradrenaline (1-[7-³H]noradrenaline hydrochloride, 5.8 Ci/mmol, The Radiochemical Centre, Amersham, UK) gassed with 5% CO₂ in O₂.

After washing in isotope-free buffer for 10 min, each vas was mounted on a perspex holder in a small (5 ml) organ bath and connected to a force-displacement transducer (Shinkoh + 20 g Type UL). Mechanical responses were recorded isometrically on a Gould chart recorder. Resting tension was adjusted to 5 x 10⁻³ Newtons and the preparation was perfused with fresh buffer for 30 min (1 ml min⁻¹). Transmural stimuli were delivered to the hypogastric nerve for 2-30 s at 30 min intervals with a Grass S44 stimulator used to generate a field between two platinum electrodes in the bath (supramaximal voltage, 2 ms duration, 20 Hz). Five stimulation periods were used in each experiment; 2 control in normal solution, 1 after 25 min in the presence of the drug being investigated and 1 each after 25 and 55 min washout periods.

The buffer was drained directly into counting vials at 5 min intervals and total radioactivity was determined in a Beckman LS100C Liquid Scintillation Counter after addition of 10 ml of Triton X100. After each experiment the vas was homogenized in 0.4 mmol 1⁻¹ perchloric acid and residual radioactivity measured as above.

Stimulation-induced tritium overflow was calculated by subtracting the estimated spontaneous overflow during the stimulation period. To allow comparison between different preparations, this value was expressed as a percentage of the total tritium content in the organ at the onset of each stimulation. This procedure corrected for the continual decline in the ³H-noradrenaline both in the tissue and in the perfusate after each stimulation (cf. Farnebo and Malmfors 1971). The percentage stimulation-induced overflow was then set to unity for the first stimulation period and successive values were expressed relative to this initial overflow.

Skeletal Neuromuscular Junction. Experiments were performed on isolated hemi-diaphragm/phrenic nerve prepara-
tions taken from adult (50 - 60 g) male mice (Füllinsdorf). Animals were stunned and bled and the muscle with its attached nerve removed and placed in a 1 – 2 ml perspex chamber continuously perfused (1 ml min⁻¹) with a modified Krebs’ solution of the following composition (mmol l⁻¹): NaCl 120; KCl 3.5; CaCl₂ 2; MgCl₂ 1; NaH₂PO₄ 1; NaHCO₃ 25; glucose 11, bubbled continuously with 5% CO₂ in O₂. When quantal content was reduced to prevent the generation of action potentials in the muscle the MgCl₂ concentration was raised to 5 mmol l⁻¹. In these solutions and in solutions containing elevated potassium concentrations isosmolarity was maintained by adjusting the concentration of NaCl.

Most experiments were conducted at room temperature (21 – 25°C) but the experimental temperature was altered when necessary by changing the temperature of fluid circulating in a jacket surrounding the organ bath.

Conventional microelectrode techniques were used to record from single end-plates in the muscle using glass micropipettes (20 – 40 MΩ resistance) filled with 3 mol l⁻¹ KCl. Signals were led from a high-impedance pre-amplifier and displayed on an oscilloscope screen and photographed or fed through an analog-to-digital converter (10 μs per point maximum sampling rate) and averaged in a Nicolet 527 signal averager or analysed in a mini-computer (Tektronix 4051).

To study evoked transmitter release the phrenic nerve was drawn into a suction electrode (Transidyne General) and stimulated with supramaximal square wave pulses from an isolated stimulator.

Drugs. Drugs used were adenosine (Sigma, St. Louis, MO, USA), 1-methyladenosine (Sigma, St. Louis, MO, USA), 2-chloroadenosine (Sigma, St. Louis, MO, USA) and 1-methylisoguanosine, either isolated from the marine sponge Tedania digitata or prepared synthetically (Quinn et al. 1980), d-tubocurarine hydrochloride (Fluka, Buchs, Switzerland), tetrodotoxin (Sankyo, Tokyo, Japan).

Results

Effects on Noradrenaline Overflow from the Guinea-Pig Vas Deferens

As a number of previous studies on the effects of purine nucleosides on transmitter release have used ³H-noradrenaline overflow as a monitor of evoked transmitter release we examined the effects of the novel nucleoside used in this study (1-methylisoguanosine) and adenosine itself on this system. This allowed comparison of the effects in this system with effects on evoked transmitter release at the skeletal neuromuscular junction measured electrically.

There was a biphasic mechanical response of the guinea-pig vas deferens to 20 s supramaximal stimuli at 20 Hz delivered to the hypogastric nerve. An initial rapid “twitch” was followed by a slow and not well maintained “tetanus” (cf. Swedin 1970). An example of this is shown in Fig. 1A. Experiments involved 5 – 6 successive stimuli separated by 30 min: during this time the stimulation-induced overflow of ³H-noradrenaline declined only marginally and the percentage overflow for these responses (see Methods) was generally well maintained, as were the mechanical responses. Each period of stimulation resulted in the release of approximately 1 – 4% of the total radioactivity present in the tissue at the onset of stimulation, the principal variations being between preparations rather than between stimuli in a single preparation.

The effects of adenosine (50 – 100 μmol l⁻¹) and 1-methylisoguanosine (50 – 100 μmol l⁻¹) on mechanical responses were minimal. Incubation of the vas deferens for 20 min with either of these nucleosides resulted in a marginal and reversible enhancement of both the amplitude and duration of the twitch response and a slight reduction in the maintenance of the “tetanic” phase of the contraction (Fig. 1A).

In the same concentrations adenosine and 1-methylisoguanosine caused a reduction in the stimulus induced overflow of ³H-noradrenaline (Fig. 1C). This effect did not reverse readily, however, and recovery to control levels was achieved in only a few experiments. In these experiments the two nucleosides were about equipotent in depressing ³H-noradrenaline overflow.