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9-Amino-1,2,3,4-tetrahydroacridine (THA) is a potent blocker of cardiac potassium channels

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1 9-Amino-1,2,3,4-tetrahydroacridine (THA) is a compound with structural similarity to the K^+ channel blocker 4-aminopyridine. It was investigated for its effects on myocardial membrane currents in guinea-pig isolated ventricular myocytes.

2 THA prolonged the transmembrane action potential and decreased the amplitude of its plateau in a reversible manner.

3 Voltage-clamp experiments showed that THA reduced the inwardly rectifying and the time-dependent outward K^+ currents as well as the slow inward Ca^{2+} current.

4 The degree of block of the inwardly rectifying K^+ current depended on the membrane potential, being more pronounced at more positive potentials.

Introduction

Potassium (K^+) channel blockers are of potential therapeutic interest in the treatment of cardiac arrhythmias. For instance, bretylium or bethanidine prolong the myocardial action potential by this mechanism (Bacaner *et al.*, 1986), which results in a prolongation of the effective refractory period. Compounds of this class (class III according to Vaughan Williams, 1975) are effective in the treatment and/or prevention of ventricular fibrillation.

The present study deals with the effect of 9-amino-1,2,3,4-tetrahydroacridine (THA) on K^+ conductance in guinea-pig isolated ventricular myocytes. It was prompted by a recent study (Summers *et al.*, 1986) which showed that THA was beneficial in the treatment of dementia of Alzheimer type. Because of a structural similarity of THA to the K^+ channel blocker, 4-aminopyridine, the authors considered that K^+ channel blockade might be involved, in addition to its anticholinesterase properties, in the cognitive improving action. The results of the present study indicate, indeed, that THA is a potent K^+ channel blocker, with additional Ca^{2+}-antagonistic properties. However, it remains to be proven whether THA would be beneficial in the treatment of ventricular fibrillation.

Methods

Adult guinea-pigs of either sex (strain, Ibm: GOHI), weighing 200–300 g, were killed by cervical dislocation and the chest opened by midline incision. The hearts were quickly removed and mounted in a Langendorff apparatus for retrograde perfusion at a pressure of 75 cmH_2O. The hearts were perfused with solutions (gassed with 95% O_2/5% CO_2 and warmed to 36°C) in the following sequence: (a) 3 min with Krebs-Henseleit solution (composition in mM: NaCl 120, KCl 5.9, $MgCl_2$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 15, glucose 11 and $CaCl_2$ 1.8); (b) 3 min with (nominally) Ca^{2+}-free Krebs-Henseleit solution; (c) 30 min recirculation with collagenase (type II, Worthington, Freehold, NJ, U.S.A; 30 mg collagenase 100 ml^{-1}) in Ca^{2+}-free Krebs-Henseleit solution; and (d) wash-out of collagenase from the heart by perfusion with 50 ml of a 'storage solution' (Isenberg & Klöckner, 1982). The 'storage solution' contained (in mM): glutamic acid 70, taurine 10, KCl 25, KH_2PO_4 10, glucose 11, EGTA 0.5 and HEPES 10 (pH adjusted with KOH to 7.3). The ventricles were then cut off and dissected into small pieces which were gently shaken in a beaker containing 'storage solution'. The resulting dissociated myocytes were filtered on a cell sieve and stored for at least 1 h in 'storage solution' at room temperature before use. This method of storage has been shown to increase the number of Ca^{2+}-tolerant (viable) cells (Isenberg & Klöckner, 1982) upon reperfusion with Ca^{2+}-containing Krebs-Henseleit solution.

Myocytes were allowed to settle on the glass bottom of a perspex recording chamber which was rapidly perfused with Krebs-Henseleit solution (37°C).

Measurement of the Ca^{2+} current (I_{Ca}) was made using the whole-cell voltage-clamp technique as described by Hamill *et al.* (1981). Patch clamp electrodes (resistance 2–3 MΩ) were pulled from Pyrex glass (Hilgenberg, Malsfeld, FRG) and filled with a solution containing (in mM): KCl 145, Na_2ATP 1, $MgCl_2$ 1 and HEPES 5 (adjusted to pH 7.4 with KOH).The electrodes were connected by a silver wire to the headstage of a potential follower (Ehrler, Homburg/Saar, FRG) in order to record the membrane potential. Action potentials were elicited by injection of depolarizing current (2–5 nA, 2 ms duration) through the recording electrode. The output of the potential follower was fed into the input of a voltage-clamp amplifier (Ehrler, Homburg/Saar, FRG). The output of the voltage-clamp amplifier was connected to a current injecting device of the potential follower, thus enabling the potential of the cell membrane to be clamped at a constant level. A command input of the voltage-clamp amplifier was connected to the output of a digital pulse generator (Ehrler, Homburg/Saar, FRG). Analog signals were displayed on a storage oscilloscope (Tektronix), stored on an 8-channel PCM tape (Heim, Bergisch-Gladbach, FRG) and analysed after digitization on a DATALAB 4000 B microprocessor system (Data Laboratories, Mitcham, UK).

THA (9-amino-1,2,3,4-tetrahydroacridine hydrochloride) was purchased from Aldrich (Steinhausen, FRG). It was prepared as a 10^{-2} M aqueous stock solution and diluted with Krebs-Henseleit solution to the final concentrations.

Results

Action potential measurements

For determination of the effects of THA on the transmembrane action potential of guinea-pig isolated ventricular myocytes the cells were stimulated regularly at 0.33 Hz, until the action potential had attained a stable configuration (usually less than a minute). A representative experiment, using a drug concentration of 10^{-4} M, is illustrated in Figure 1a. After the perfusate had been changed, loss of action potential plateau and a drastic action potential prolongation were observed. Due to the small volume of the perfusion chamber (~0.3 ml) and a rapid perfusion (1–2 ml min^{-1}), the steady-state drug effect was reached within less than 2 min. In this particular experiment, action potential amplitude decreased by 15 mV, and its duration at the 90% repolarization level increased from 180 to 250 ms. On wash-out of the drug, the effects reversed completely (and with a similar time course to their onset).

In the experiment demonstrated in Figure 1a, there was no effect on the membrane resting potential.

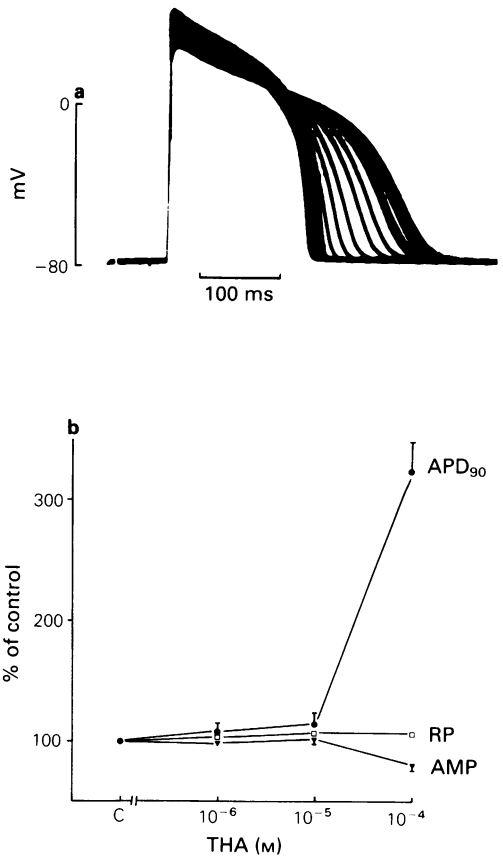

Figure 1 Effects of tetrahydroaminoacridine (THA) on the transmembrane action potential of guinea-pig isolated myocytes. (a) Shows superimposed action potentials recorded continuously from a myocyte which was regularly stimulated every 3 s. THA, administered to the cell in a concentration of 10^{-4} M, caused a prolongation of the action potential and a decrease of its amplitude. The shortest action potential was still recorded under drug-free conditions. Note the rapid development of the effect of THA. (b) Concentration-effect relationship for the actions of THA on the action potential. APD_{90} indicates duration at 90% repolarization. AMP stands for the action potential amplitude and RP for the resting membrane potential. The data are expressed as % of control (C, = 100%); each value is the mean obtained from 4 cells; vertical lines indicate s.e.mean.

However, in 1 out of 5 experiments with isolated myocytes the resting potential fell to an ultimate level of about −20 mV within about 5 min. Such an observation was not made in three further experiments using guinea-pig isolated papillary muscles (data not shown). Considering the high drug concentration

(10^{-4}M) and the solitary occurrence, a therapeutic relevance is improbable.

Figure 1b summarizes the effects of THA, 10^{-6}, 10^{-5} and 10^{-4}M, on resting membrane potential, action potential amplitude and action potential duration at 90% repolarization, on 4 isolated myocytes (excluding the cell where depolarization to −20 mV had occurred). THA had apparently no effect on the resting potential. The action potential amplitude was significantly decreased only at 10^{-4}M. The concentration-response curve for prolongation of the action potential duration was surprisingly steep, with only a 14 ± 9% increase at 10^{-5}M but 323 ± 24% increase at 10^{-4}M THA.

Voltage clamp studies

In one series of experiments, a protocol with voltage-clamp pulses was used to study the effects of THA on the membrane currents. The membrane potential of the cell was held at −50 mV to inactivate the Na^+ current and transiently depolarized for either 200 or 500 ms to +10 mV or +20 mV at a frequency of 0.33 Hz. Figure 2a shows recordings of the current in response to a 200 ms clamp pulse in the steady-state before and during administration of 3 different concentrations of THA (10^{-6}, 10^{-5} and 10^{-4}M). The following observations were made: (1) THA concentration-dependently decreased the amplitude of the holding current at −50 mV. This current component corresponds to the time-independent, outwardly directed, inwardly rectifying K^+ current. Inhibition (determined in 3 experiments) was negligible at 10^{-6}M (2 ± 2%; mean ± s.e.mean) and amounted to 18 ± 4% at 10^{-5} and 72 ± 7% at 10^{-4}M. (2) The negative surge of current after the onset of the clamp pulse (from −50 mV holding potential) corresponds to the rapidly activating and then more slowly inactivating Ca^{2+} inward current. THA concentration-dependently decreased this current and nearly abolished it at 10^{-4}M. (3) The slowly activating time-dependent ouward current, which becomes visible during the later phase of the voltage-clamp pulse, was reduced in a similar concentration-dependent manner. This effect is more marked with longer test pulses (e.g. 500 ms) to more depolarized levels (e.g. +20 mV), as shown in Figure 2b.

An interesting aspect of the action of the aminopyridines on K^+ channels is the voltage-dependence of the block (Yeh *et al.*, 1976). The amount of block was found to be substantially less marked, the more positive the membrane depolarizations or the longer the depolarizations at a given depolarized potential. I investigated a possible potential-dependence of the THA effect by measuring the steady-state current-voltage relationship. To determine this, the membrane potential was clamped to −130 mV and then depolarized in 10 mV steps up to +10 mV. The duration of application of each potential was sufficiently long (around 10 s) to allow the membrane current to reach a steady state. The results of such an experiment are depicted in Figure 3. The current-

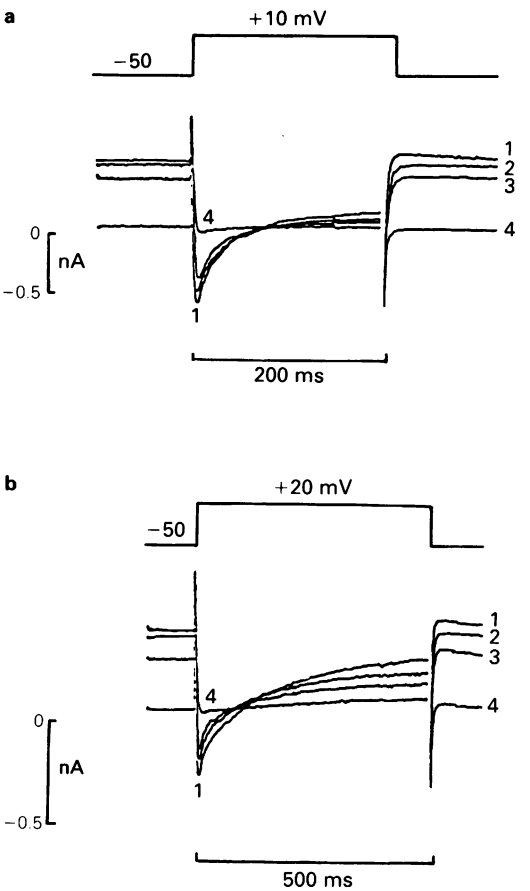

Figure 2 Effects of tetrahydroaminoacridine (THA) on voltage-clamped myocytes. The membrane potential of a myocyte was voltage-clamped at a holding potential of −50 mV and transiently depolarized for either 200 ms (in a) or 500 ms (in b) to +10 mV (in a) or +20 mV (in b) every 3 s (the clamp steps are indicated schematically at the top of each figure). The traces depicting the current recorded before THA administration are labelled 1, those recorded in the steady-state in the presence of 10^{-6}, 10^{-5} and 10^{-4}M THA are labelled 2, 3 and 4, respectively. THA was applied cumulatively, and steady-states were reached within about 2 min. Note that the holding current at −50 mV becomes progressively less negative, and that both the slow inward Ca^{2+} current and the time-dependent K^+ outward currents are depressed with increasing drug concentrations.

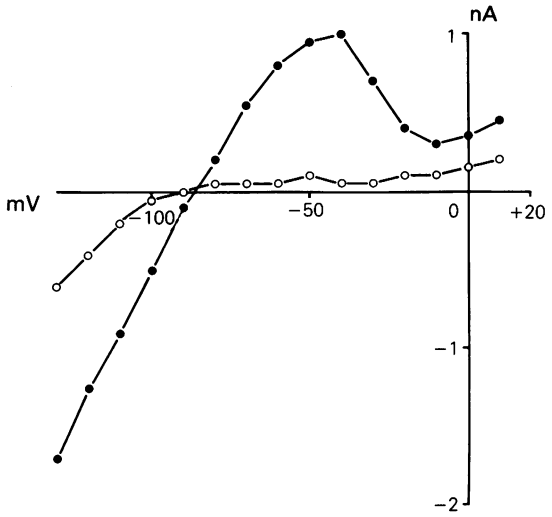

Figure 3 Steady-state current-voltage relationship of the outwardly directed, inwardly rectifying K^+ current measured before (●) and in the presence of (○) 10^{-4} M tetrahydroaminoacridine.

voltage relationship in drug-free perfusion medium displays the typical N-shape, which is characterized by a relatively small current amplitude around 0 mV, as compared to − 50 mV. Such a shape has been described in various cardiac preparations and species (for references see Trautwein, 1973), including guinea-pig papillary muscle (Ochi, 1970) and guinea-pig isolated ventricular myocytes (Sakmann & Trube, 1984). THA, administered at a concentration of 10^{-4} M, had a pronounced effect on the steady-state current voltage relationship. At values more positive than − 80 mV (which is the approximate K^+ reversal potential), the curve became nearly flat. At values more negative than − 80 mV, the curve became less steep in the presence of THA. However, on a percentage basis the effect appeared attenuated at potentials negative to E_K. This result was confirmed in 3 other cells. Thus, the THA-induced block of the inwardly rectifying K^+ channel depends on the membrane potential, albeit in a manner inverse to that of 4-aminopyridine.

Discussion

The purpose of the present paper was to show whether THA has K^+ channel blocking properties on guinea-pig myocytes. Inhibition of both the inwardly rectifying and the delayed outward K^+ current was directly shown in voltage-clamp experiments. This inhibition results in a prolongation of the transmembrane action potential, which may be of potential relevance in developing novel antiarrhythmic drugs of the class III type. Of further pharmacological interest is the ability of THA to block the Ca^{2+} channels. The structurally related 4-aminopyridine had virtually no blocking effect on the Ca^{2+} current in guinea-pig ventricular myocytes (data not shown), which is in accordance with its lack of effect on the Ca^{2+} current in sheep cardiac Purkinje fibres (Kenyon & Gibbons, 1979).

Although neuronal preparations have not been investigated in this study, it seems possible that a similar block of potassium channels by THA may occur in neurones, especially since other potassium channel blockers such as 4-aminopyridine are known to exert widespread effects on different cell types (for review, see Soni & Kam, 1982).

THA was more potent in my experiments than 4-aminopyridine; THA 10^{-4} M reduced the holding current at − 50 mV by 72% whereas 4-aminopyridine reduced it by only 6 ± 3% ($n = 4$; data not shown). In sheep cardiac Purkinje fibres, 4-aminopyridine was without effect on the inward rectifying current (Kenyon & Gibbons, 1979). Two other K^+ channel blockers have been described, that are chemically related to 4-aminopyridine, but more potent: 3,4-diaminopyridine (Kirsch & Narahashi, 1978) and 4-aminoquinoline (Guerrero & Zacharias, 1984). Thus, it seems possible that chemical modifications of THA (or 4-aminopyridine) may result in still more potent K^+ channel blockers.

The potential dependence of K^+ channel block by THA appears at present to be mainly of theoretical interest. I restrict myself to the discussion of one aspect. The pK_a of acridine dyes, such as proflavin, is in the same order or even larger than that of 4-aminopyridine ($pK_a = 9.18$). Therefore, their uptake into the cell, at physiological pH, is very slow (Robbins, 1960). The rapid development of block by THA could be explained by an interaction at a binding site (or sites) at the external side of the cell membrane, in contrast to 4-aminopyridine which is believed to act from the inside of the cell (Hermann & Gorman, 1981). This might explain a different dependence of block on the direction of current flow.

In conclusion, THA is a K^+ channel blocker with interesting properties as a pharmacological and physiological tool.

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References

BACANER, M.B., CLAY, J.R., SHRIER, A. & BROCHU, R.M. (1986). Potassium channel blockade: A mechanism for suppressing ventricular fibrillation. *Proc. natn. Acad. Sci. U.S.A.* **83,** 2223–2227.

GUERRERO, S. & ZACHARIAS, J. (1984). Electrophysiological effects of 4-aminoquinoline on frog atrial contractile fibers. *Arch. int. Pharmacodyn. Ther.*, **269,** 94–110.

HAMILL, O.P., MARTY, A., NEHER, E., SAKMANN, B. & SIGWORTH, F.J. (1981). Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Arch.*, **391,** 85–100.

HERMANN, A. & GORMAN, A.L.F. (1981). Effects of 4-aminopyridine on potassium currents in a molluscan neuron. *J. gen. Physiol.*, **78,** 63–86.

ISENBERG, G. & KLÖCKNER, U. (1982). Calcium tolerant ventricular myocytes prepared by incubation in a "KB" medium. *Pflügers Arch.*, **395,** 6–18.

KENYON, J.L. & GIBBONS, W.R. (1979). 4-Aminopyridine and the early outward current of sheep cardiac Purkinje fibers. *J. gen. Physiol.*, **73,** 139–157.

KIRSCH, G.E. & NARAHASHI, T. (1978). 3,4-Diaminopyridine. A potent new potassium channel blocker. *Biophys. J.*, **22,** 507–512.

OCHI, R. (1970). The slow inward current and the action of manganese ions in guinea pig's myocardium. *Pflügers Arch.*, **316,** 81–94.

ROBBINS, E. (1960). The rate of proflavin passage into single living cells with application to permeability studies. *J. gen. Physiol.*, **43,** 853–866.

SAKMANN, B. & TRUBE, G. (1984). Conductance properties of single inwardly rectifying potassium channels in ventricular cells from guinea-pig heart. *J. Physiol.*, **347,** 641–657.

SONI, N. & KAM, P. (1982). 4-Aminopyridine – a review. *Anaesth. Intens. Care*, **10,** 120–126.

SUMMERS, W.K., MAJOVSKY, L.V., MARSH, G.M., TACHIKI, K. & KLING, A. (1986). Oral tetrahydroaminoacridine in long-term treatment of senile dementia, Alzheimer type. *New Engl. J. Med.*, **315,** 1241–1287.

TRAUTWEIN, W. (1973). Membrane currents in cardiac muscle fibers. *Physiol. Rev.*, **53,** 793–835.

VAUGHAN WILLIAMS, E.M. (1975). Classification of antidysrhythmic drugs. *Pharmac. Ther. B.*, **1,** 115–138.

YEH, J.Z., OXFORD, G.S., WU, C.H. & NARAHASHI, T. (1976). Interactions of aminopyridines with potassium channels of squid axon membranes. *Biophys. J.*, **16,** 77–81.

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