Different time courses of the blockade of sodium current by lignocaine and SUN 1165 in single myocytes isolated from guinea-pig atrium

Norio Inomata,¹Takafumi Ishihara & *Norio Akaike

Laboratory of Molecular Pharmacology, Suntory Institute for Biomedical Research, Shimamoto-cho, Mishima-gun, Osaka 618 and *Department of Neurophysiology, Tohoku University School of Medicine, Sendai 980, Japan

1 The time course of the blockade of sodium currents (I_{Na}) by the antiarrhythmic agents, lignocaine and SUN 1165, was studied in single myocytes isolated enzymatically from guinea-pig atrium, by a new concentration-jump termed as a 'concentration-clamp' technique. This technique combines an intracellular perfusion and a rapid exchange of external solution surrounding the voltage-clamped myocyte within 2 ms.

2 Lignocaine (3.7×10^{-5} M to 3.7×10^{-4} M) inhibited the peak amplitude of I_{Na} in a concentration-dependent fashion. It took 2 to 5 s to reach apparent steady-state inhibition at the concentrations used. Complete recovery from the inhibition also took 2 to 5 s after washing out the agent. In contrast, the inhibitory effect of SUN 1165 (1×10^{-5} M) on the peak I_{Na} gradually progressed and reached a steady-state level about 2 min after the start of drug-application. The recovery required more than 10 min after washing out of the agent.

3 In cardiomyocytes treated with scorpion toxin ($5 \mu\text{g ml}^{-1}$), the inactivation of I_{Na} was greatly inhibited, resulting in the relatively persistent Na inward current (persistent I_{Na}) during the depolarizing pulse. Lignocaine (1.1×10^{-4} M) applied during the depolarizing pulse, reduced in a single-exponential fashion the amplitude of the persistent I_{Na} in milliseconds. On the other hand, lignocaine applied several tens of milliseconds before the depolarizing pulse induced only a small reduction of the peak amplitude of the first persistent I_{Na} . When SUN 1165 (1×10^{-5} M) was applied during the depolarizing pulse, there was only a small instantaneous reduction of the amplitude of the persistent I_{Na} , although it did inhibit time-dependently, the peak I_{Na} . Both agents accelerated the decay phase of the persistent I_{Na} in a time-dependent manner.

4 These results suggest that lignocaine and SUN 1165 may preferentially interact with the open-state of the sodium channel rather than with the rested one, although SUN 1165 does so much more slowly.

Introduction

N-(2,6-dimethylphenyl)-8-pyrrolizidine acetamide hydrochloride hemihydrate (SUN 1165), has been demonstrated to possess a potent antiarrhythmic action in *in vivo* and *in vitro* studies (Aisaka *et al.*, 1985; Hidaka *et al.*, 1985; Inomata & Ishihara, 1988). SUN 1165 bears a structural resemblance to lignocaine, but it has a special feature (pyrrolizidine) which is not present in commonly used antiarrhythmic agents (Miyano *et al.*, 1985). Voltage-

clamp studies with isolated single cardiomyocytes have clearly shown that the effects of SUN 1165 on the sodium current (I_{Na}) resemble those of lignocaine and that its blocking action on the I_{Na} is the main mechanism of antiarrhythmic action (Yatani & Akaike, 1984; Inomata *et al.*, 1987). There may be a common basis for the mechanism of action of both antiarrhythmics that involves a voltage- and use-dependent inhibition of sodium channels (Class I-type), although they have been classified as different subtypes in terms of onset and offset kinetics of

¹ Author for correspondence.

Figure 1 Time-course of the blocking action of I_{Na} and the recovery from the blockade of I_{Na} by lignocaine in single guinea-pig atrial cells. (a) The superimposed original I_{Na} traces after the application (left) and the washing out (right) of 1.1×10^{-4} M lignocaine. Each number shown at the right side of the current traces indicates the time (s) after the application or the washing out of the agent. (b) The time courses of the inhibitory action of lignocaine on peak I_{Na} after the application and the washing out of several concentrations of the agent: (○) 3.7×10^{-5} M; (□) 1.1×10^{-4} M; (△) 3.7×10^{-4} M. Each symbol indicates the mean of five experiments. Amplitude of peak I_{Na} was regarded as the difference between the maximum peak level and the terminal level.

the blocking action on the maximum rate of rise of the action potential (Campbell, 1983; Pallandi & Campbell, 1988; Hattori *et al.*, 1986; 1988).

The introduction of a 'concentration-clamp' technique which combines intracellular perfusion and a rapid solution change with a time constant of a few ms for the cell-attached condition of isolated cells has made it possible to analyse the precise time course of drug action and the interaction between the ionic channels (open and closed states) and drugs (Akaike *et al.*, 1986; Oyama *et al.*, 1987; 1988; Inomata *et al.*, 1988). Thus, it is now possible to estimate accurately the changes of sodium ion permeability induced by antiarrhythmic agents occurring in the millisecond order. Consequently, in order to characterize more precisely the inhibitory action of SUN 1165 on the sodium channels of car-

diomyocytes, a comparison of the effects on I_{Na} between lignocaine and SUN 1165 was made by use of the 'concentration-clamp' technique.

Methods

The enzymatic dispersal procedure used for preparation of single atrial myocytes was essentially similar to that previously described (Matsuda *et al.*, 1982). In brief, the hearts of male guinea-pigs weighing 300 to 500 g were used. The aorta was cannulated and the heart was perfused with nominally Ca^{2+} -free Tyrode solution until the spontaneous contractions stopped. Thereafter, the perfusate was changed to the Ca^{2+} -free solution containing 0.4 mg ml^{-1} collagenase (Sigma, Type I) for about 45 min. Then the enzyme was washed out with a high-K storage solution composed of (in mM): KCl 85, K_2HPO_4 30, $MgSO_4$ 5, Na_2ATP 5, pyruvate 5, creatine 5, taurine 20, glucose 20, EGTA 0.2. The pH of the storage solution was adjusted to 7.4. The temperature of all solutions was kept at 37°C . The right atrium was dispersed in the storage solution with forceps. The dispersed single myocytes were left in the storage solution for later use at room temperature of 24 to 26°C .

Experiments were performed on viable rod-shaped myocytes with a 'concentration-clamp' technique (Akaike *et al.*, 1986). A single myocyte was sucked onto the tip of a suction pipette (inner diameter of 2 to $3 \mu\text{m}$ at the tip) and dialysed intracellularly with the internal solution (pH 7.4) containing (in mM): KOH 130, L-aspartate 130, KH_2PO_4 2.5, Na_2ATP 2, pyruvate 5, glucose 5.5 and HEPES 10. The tip of the suction pipette with the cell attached was inserted into a plastic tube through a circular hole (see Figure 1 of Akaike *et al.*, 1986). The lower end of this tube could be directly exposed to the external solution by vertically moving a stage holding the dishes containing solutions (see Figure 1 of Akaike *et al.*, 1986). The composition of the external solution (pH 7.4) was as follows (in mM): NaCl 137, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.53, NaH_2PO_4 0.05, $NaHCO_3$ 11.9, glucose 5.5, HEPES 5. A negative pressure of -3 cmHg applied to the upper end of the plastic tube was controlled by an electromagnetic valve, driven by 24 V d.c. supplied by a stimulator. The time constant of the solution exchange around an atrial myocyte was about 2 ms.

The ionic current was recorded by a single electrode voltage-clamp system (Ishizuka *et al.*, 1984). Both membrane current and potential were monitored on a digital oscilloscope (National, VP-5730A) and simultaneously stored on the FM-tape recorder (Sony, A-45) for further analysis. All experiments

were carried out at a room temperature of 24 to 26°C. The drugs used were SUN 1165 (Suntory), lignocaine (Sigma) and scorpion toxin (from *Leiurus quinquestriatus*, Sigma). All drugs were dissolved in the external solution just before use.

Results

Effect on the sodium current

The sodium current (I_{Na}) was elicited by a depolarizing pulse, lasting for 20 ms, from a holding potential of -80 mV to -20 mV at a frequency of 1 Hz. No use-dependent changes in the I_{Na} were observed at this stimulus frequency. Lignocaine was applied to voltage-clamped single myocytes 800 ms before the depolarizing pulse. As shown in Figure 1, the inhibition of the peak amplitude of I_{Na} by lignocaine rapidly progressed at any concentration used in this study and reached a steady-state level within 2 to 5 s after the drug application. The degree of inhibition on the peak amplitude of I_{Na} became more profound as the concentration of lignocaine was increased. The peak amplitudes of I_{Na} at the steady-state inhibition, relative to the control, were 0.94 ± 0.04 (mean $\pm$ s.e. of five experiments) at 3.7×10^{-5} M lignocaine, 0.71 ± 0.06 at 1.1×10^{-4} M and 0.39 ± 0.07 at 3.7×10^{-4} M. The recovery from the inhibition was complete within 2 to 5 s after washing out of lignocaine at any concentration used. In contrast to lignocaine, SUN 1165 was slow in inhibiting the peak amplitude of I_{Na} and it took 2 min or more to reach the steady-state inhibition as shown in Figure 2. The concentration (1×10^{-5} M) of SUN 1165 used in this study was close to the maximum value of effective plasma concentrations required for terminating ventricular arrhythmia in dogs (Ishihara *et al.*, 1984). Complete recovery from the inhibition was also observed, but it required more than 10 min after washing out the agent.

Effect on the persistent sodium current

The action of a drug that shortens the time to peak and hastens the decay phase has been accounted for by a model in which a drug blocks the open-state of channels (Yeh & Narahashi, 1977). However, the decay phase of I_{Na} is too rapid to make an exact evaluation of drug interaction with the open-state of Na channels. Scorpion toxin is known to prolong the decay phase of I_{Na} without affecting the sodium current activation kinetics in nerve axon (Narahashi *et al.*, 1972). Therefore, single atrial myocytes were treated with scorpion toxin ($5 \mu\text{g ml}^{-1}$) for about 3 min in order to produce a relatively-persistent sodium current (persistent I_{Na}). In the subsequent

Figure 2 Time-course of inhibitory action of SUN 1165 on the peak I_{Na} in single guinea-pig atrial cells. (a) The superimposed original I_{Na} traces after the application (left) and the washing out (right) of 1×10^{-5} M SUN 1165. Each number shown at the right side of the current traces indicates the time (s) after the application or the washing out of the agent. (b) The time course of inhibitory action of 1×10^{-5} M SUN 1165 after the application and the washing out of the agent.

experiments, the persistent I_{Na} was elicited by applying a depolarizing pulse of 150 ms in duration to -25 mV from a holding potential of -85 mV every 5 s (Figure 3a). There were no use-dependent changes in the current under these conditions.

The persistent I_{Na} induced in this way, was suddenly deflected from its predicted decay time course when lignocaine at a concentration of 1.1×10^{-4} M was applied to the preparation during the depolarizing pulse as shown in Figure 3b. Thus, there was no detectable delay in onset of the inhibitory action of lignocaine. The reduction in the current amplitude of persistent I_{Na} by 1.1×10^{-4} M lignocaine progressed in a single-exponential fashion with the time constant of 39 ± 2 ms (mean $\pm$ s.e. of eight experiments). The deflection of the current by lignocaine became more pronounced as the concentration was increased and the time constant of the reduction in the amplitude of the persistent I_{Na} also decreased in a concentration-dependent manner (not shown). When 1.1×10^{-4} M lignocaine was applied during

Figure 3 (a) The inhibition of sodium channel inactivation by scorpion toxin in a single atrial cell of guinea-pig. Original current trace in the control (left) and in the presence of scorpion toxin $5 \mu\text{g ml}^{-1}$ (right). (b) The inhibitory action of $1.1 \times 10^{-4} \text{ M}$ lignocaine on the I_{Na} of scorpion toxin-treated single atrial cells. Current traces show the control (upper), effect of lignocaine applied during the depolarizing pulse (middle), and the second and third pulses after the application of lignocaine (lower), respectively. (c) The inhibitory action of $1 \times 10^{-5} \text{ M}$ SUN 1165 on the I_{Na} of the scorpion toxin-treated single atrial cells. Current traces show the control (upper), effect of SUN 1165 applied during the depolarizing pulse (middle), and the second to 9th pulses after the application of the agent (lower), respectively. The outward deflection indicated by the asterisk occurred because of no correction for the leakage current.

the depolarizing pulse, the amplitude of the persistent I_{Na} at 30 and 80 ms after the application of the drug, relative to control, was 0.51 ± 0.02 and 0.17 ± 0.01 (mean $\pm$ s.e. of four experiments), respectively. On the other hand, when $1.1 \times 10^{-4} \text{ M}$ lignocaine was applied 30 and 80 ms before the depolarizing pulse, the peak amplitudes of the first persistent I_{Na} were 0.96 ± 0.01 and 0.93 ± 0.04 (mean $\pm$ s.e. of four experiments), respectively. The steady-state inhibition by $1.1 \times 10^{-4} \text{ M}$ lignocaine was observed from the 2nd depolarizing pulse. The

decay phase of persistent I_{Na} was greatly accelerated in the continued presence of lignocaine (Figure 3b). The half decay time decreased from 41 ms to 3 ms when the drug effect reached a steady-state.

SUN 1165 at a concentration of $1 \times 10^{-5} \text{ M}$ applied during the depolarizing pulse affected only slightly the amplitude of the persistent I_{Na} (Figure 3c). However, the agent again slowly reduced the peak current (see Figure 2). The inhibition of the persistent I_{Na} by SUN 1165 was also associated with an accelerated decay phase of the current. In the continued presence of $1 \times 10^{-5} \text{ M}$ SUN 1165, the time for half decay of the persistent I_{Na} decreased from 40 ms in control to 10 ms at the 10th depolarizing pulse (about 45 to 50 s after the start of drug-application).

Discussion

In the present study, we found that both lignocaine and SUN 1165 produced a final reduction of the peak amplitude of I_{Na} in single cardiomyocytes, but the time courses to a steady-state in their inhibitory actions were quite different from each other (compare Figure 1 and 2). Lignocaine blocked the peak I_{Na} in the order of seconds while SUN 1165 required minutes when both agents were applied to the preparation before a depolarizing pulse. Similarly although the recovery from inhibition was complete in both cases, the time required for recovery from the inhibition was again in seconds for lignocaine and minutes for SUN 1165. These results suggest that the rates of binding to and release from the sodium channels are much slower for SUN 1165 than for lignocaine.

In order to know more certainly whether lignocaine and SUN 1165 block open Na channels, we used scorpion toxin to keep the channels open for a considerably longer time. We succeeded in recording a persistent I_{Na} from cardiomyocytes treated with scorpion toxin (Figure 3a) and examined the effects of these drugs on it. We found that the rate of inhibition by lignocaine occurred in a few milliseconds when the agent was applied during the depolarizing pulse, which was not the case for SUN 1165 (Figure 3). The prompt acceleration of the rate of inhibition of the persistent I_{Na} by lignocaine is reminiscent of the rapid interaction between the drug and open sodium channels and indicates that lignocaine preferentially blocks the open state of Na channels rather than the rested one because the time course of the inhibitory action of the agent, as shown in Figure 1 and also in scorpion toxin-treated cells, is much slower when the agent is applied before the depolarizing pulse. In contrast, SUN 1165 had almost no instantaneous effect on the persistent I_{Na} ,

although, in the continued presence of SUN 1165, the decay phase of persistent I_{Na} was greatly accelerated when the drug effect on the peak amplitude of the persistent I_{Na} reached the steady state. These results suggest that SUN 1165 also blocks the open state of sodium channels.

However, it may be argued that both lignocaine and SUN 1165 cause a simple displacement of scorpion toxin from its binding site which results in an apparent acceleration of the decay phase of I_{Na} . This seems unlikely because Class I-type antiarrhythmic agents such as procainamide do not inhibit the sea anemone toxin binding to the sodium channels (Sheldon *et al.*, 1987) to which scorpion toxin also binds (Catterall & Beress, 1978). It is also possible that scorpion toxin makes another channel carry the additional inward current. This is also unlikely because the membrane potential at which the maximum peak current amplitude was produced and the reversal potential does not change in the presence of scorpion toxin (Narahashi *et al.*, 1972). Thus, the marked acceleration of the rate of inhibition of the persistent I_{Na} by lignocaine as well as by

SUN 1165 is undoubtedly due to the blockade of open sodium channels by these agents.

In general, the clinically useful antiarrhythmic agents have a low affinity for the rested state of sodium channels, while they possess a higher affinity for the open or inactivated state (Hondeghe & Katzung, 1984). An inhibitory action of both lignocaine and SUN 1165 on the inactivated state of sodium channels was previously demonstrated in isolated single cardiomyocytes (Lee *et al.*, 1981; Yatani *et al.*, 1984; Inomata *et al.*, 1987). From the present results which were obtained by applying a 'concentration-clamp' technique that made it possible to analyse the precise time course of drug action, we suggest that both lignocaine and SUN 1165 bind preferentially to the open rather than the rested state of the sodium channel although SUN 1165 does so slowly.

The authors wish to thank Drs Y. Oyama and A. Yatani for their helpful suggestions on the manuscript and Dr T. Noguchi, the director of Suntory Institute, for his support and encouragement throughout this study.

References

- AISAKA, K., HIDAKA, T., INOMATA, N., HAMASAKI, S., ISHIHARA, T. & MORITA, M. (1985). N-(2,6-dimethylphenyl)-8-pyrrolizidineacetamide hydrochloride hemihydrate (SUN 1165): A new potent and long-acting antiarrhythmic agent. *Arzneim. Forsch/Drug. Res.*, **35** (II), 1239–1245.
- AKAIKE, N., INOUE, M. & KRISHTAL, O.A. (1986). 'Concentration-clamp' study of γ -aminobutyric-acid-induced chloride current kinetics in frog sensory neurones. *J. Physiol.*, **379**, 171–185.
- CAMPBELL, T.J. (1983). Kinetics of onset of rate-dependent effects of Class-I antiarrhythmic drugs are important in determining their effects on refractoriness in guinea-pig ventricle, and prove a theoretical basis for their subclassification. *Cardiovasc. Res.*, **17**, 344–352.
- CATTERALL, W.A. & BERESS, L. (1978). Sea anemone toxin and scorpion toxin share a common receptor site associated with the action potential sodium ionophore. *J. Biol. Chem.*, **253**, 7393–7396.
- HATTORI, Y., HIDAKA, T., AISAKA, K., SATOH, F. & ISHIHARA, T. (1988). Effect of SUN 1165, a new potent antiarrhythmic agent, on the kinetics of the rate-dependent block of Na channel and ventricular conduction of extrasystoles. *J. Cardiovasc. Pharmacol.*, **11**, 407–412.
- HATTORI, Y., INOMATA, N., AISAKA, K. & ISHIHARA, T. (1986). Electrophysiological actions of N-(2,6-dimethylphenyl)-8-pyrrolizidine acetamide hydrochloride hemihydrate (SUN 1165), a new antiarrhythmic agent. *J. Cardiovasc. Pharmacol.*, **8**, 998–1002.
- HIDAKA, T., HAMASAKI, S., AISAKA, K., ISHIHARA, T., MORITA, M., TOYAMA, J. & YAMADA, K. (1985). N-(2,6-dimethylphenyl)-8-pyrrolizidineacetamide hydrochloride hemihydrate (SUN 1165), a new antiarrhythmic agent: Effects on cardiac conduction. *Arzneim. Forsch/Drug. Res.*, **35**, 1381–1386.
- HONDEGHEM, L.M. & KATZUNG, B.G. (1984). Antiarrhythmic agents: The modulated receptor mechanism of action of sodium and calcium channel blocking drugs. *Ann. Rev. Pharmacol. Toxicol.*, **24**, 387–423.
- INOMATA, N., ISHIHARA, T. & AKAIKE, N. (1987). SUN 1165: A new antiarrhythmic Na current blocker in ventricular myocytes of guinea-pig. *Comp. Biochem. Physiol.*, **87C**, 237–243.
- INOMATA, N. & ISHIHARA, T. (1988). Mechanism of inhibition by SUN 1165, a new Na channel blocking antiarrhythmic agent, of cardiac glycoside-induced triggered activity. *Eur. J. Pharmacol.*, **145**, 313–322.
- INOMATA, N., ISHIHARA, T. & AKAIKE, N. (1988). Effects of diuretics on GABA-gated chloride current in frog isolated sensory neurones. *Br. J. Pharmacol.*, **93**, 679–683.
- ISHIHARA, T., AISAKA, K., HIDAKA, T., INOMATA, N., HAMASAKI, S. & MORITA, M. (1984). N-(2,6-dimethylphenyl)-8-pyrrolizidine acetamide hydrochloride hemihydrate (SUN 1165): a new potent long-acting antiarrhythmic agent. In *Proceedings of the IUPHAR 9th International Congress of Pharmacology*, p. 458. London: Macmillan Press.
- ISHIZUKA, S., HATTORI, K. & AKAIKE, N. (1984). Separation of ionic currents in the somatic membrane of frog sensory neurons. *J. Membr. Biol.*, **78**, 19–28.
- LEE, K.S., HUME, J.R., GILES, W. & BROWN, A.M. (1981). Sodium current depression by lidocaine and quinidine in isolated ventricular cells. *Nature*, **291**, 325–327.
- MATSUDA, H., NOMA, A., KURACHI, Y. & IRISAWA, H. (1982). Transient depolarization and spontaneous voltage fluctuations in isolated single cells from guinea-

- pig ventricles. Calcium-mediated membrane potential fluctuations. *Circ. Res.*, **51**, 142–151.
- MIYANO, S., SUMOTO, K., NOGUCHI, T., SATOH, F., SHIMA, K., HAYASHIMATSU, M. & MORITA, M. (1985). New antiarrhythmic agents, N-aryl-8-pyrrolizidine acetamide. *J. Med. Chem.*, **28**, 714–717.
- NARAHASHI, T., SHAPIRO, B.I., DEGUCHI, T., SCUKA, M. & WANG, C.M. (1972). Effects of scorpion venom on squid axon membranes. *Am. J. Physiol.*, **222**, 850–857.
- OYAMA, Y., HORI, N., TOKUTOMI, N. & AKAIKE, N. (1987). D-600 blocks open Ca channels more profoundly than closed ones. *Brain Res.*, **417**, 143–147.
- OYAMA, Y., SADOSHIMA, J., TOKUTOMI, N. & AKAIKE, N. (1988). Some properties of inhibitory action of lidocaine on the Ca^{2+} current of single isolated frog sensory neurons. *Brain Res.*, **442**, 223–228.
- PALLANDI, R.T. & CAMPBELL, T.J. (1988). Selective depression of conduction of premature action potentials in canine Purkinje fibres by class Ib antiarrhythmic drugs. Comparison with Ia and Ic drugs. *Cardiovasc. Res.*, **22**, 171–178.
- SHELDON, R.S., CANNON, N.J. & DUFF, H.J. (1987). A receptor for type I antiarrhythmic drugs associated with rat cardiac sodium channels. *Circ. Res.*, **61**, 492–497.
- YATANI, A. & AKAIKE, N. (1984). Effects of a new antiarrhythmic compound SUN 1165 [N-(2,6-dimethylphenyl)-8-pyrrolizidineacetamide hydrochloride] on the sodium currents in isolated single ventricular cells. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **326**, 163–168.
- YEH, J.Z. & NARAHASHI, T. (1977). Kinetic analysis of pancuronium interaction with sodium channels in squid axon membranes. *J. Gen. Physiol.*, **69**, 293–323.

(Received February 1, 1989

Revised April 11, 1989

Accepted April 28, 1989)