Properties of Aconitine-Modified Sodium Channels in Single Cells of Mouse Ventricular Myocardium

B. NILIUS, W. BOLDT and K. BENNDORF

Julius Bernstein Institute of Physiology, Martin Luther University Halle-Wittenberg, Leninallee 6, 4020 Halle (Saale), GDR

Abstract. Effects of the plant alkaloid Aconitine on the kinetics of sodium channels were studied in enzymatically isolated single cells of the mouse ventricular myocardium. Aconitine (1 μmol/l) induced a prolongation of the 90 % repolarization of action potentials from 52.4 ± 3.7 ms to 217.0 ± 12.5 ms. Delayed terminal repolarization and oscillatory afterpotentials preceded spontaneous activity with high frequencies. Peak sodium currents were diminished from 28.0 ± 9.0 to 14.0 ± 6.0 nA. The reversal potential of the sodium current was shifted from 16.0 ± 11.0 to −8.0 ± 6.0 mV (52.5 mmol/l extracellular sodium concentration) suggesting a decreased selectivity of the Aconitine-modified Na channels. The m_∞-curves were shifted 31 mV towards more negative potentials at a constant slope. The h_∞-curves were shifted in the same direction by 13 mV. The slope parameter of the h_∞-voltage relationship was enlarged from 9.1 ± 2.2 mV to 15.6 ± 4.4 mV. Shifts in m_∞ and h_∞ resulted in an increased "window". The alkaloid modified channels inactivated extremely slowly at potentials negative to −40 mV, but showed a fast and complete inactivation at potentials positive to −40 mV.

Key words: Mouse — Myocardium — Single cells — Voltage clamp — Sodium channels — Aconitine — Arrhythmia

Introduction

In heart muscle research the alkaloid Aconitine from the plant *Aconitium napellus* has been widely used to induce experimental tachyarrhythmias (Scherf and Schott 1973; for a review, see Honerjäger 1982; Katzung 1982). Changes in action potentials due to Aconitine are quite uniform on several myocardial preparations and they consist of a substantial delay in the final repolarization phase which in turn initiates premature or triggered excitations (Matsuda et al. 1959; Schmidt 1960; Heistracher and Pillat 1962; Goto et al. 1963, 1967; Honerjäger and Meissner 1983). However, although it has been supposed that in heart muscle Aconitine

affects mainly the inactivation process of the sodium channels (Peper and Trautwein 1967), a detailed analysis of the properties of Aconitine-modified sodium channels in single cells of mammalian myocardium is not available.

In frog myocardium a detailed analysis of Aconitine induced changes in the kinetics of Na current has already been described by the use of the double sucrose gap technique (Jurevichius and Rosenstraukh 1982). The present paper concerns the kinetic properties of Aconitine-modified sodium channels in isolated cells of the mouse ventricular myocardium. Here we report a kinetic effect of Aconitine on sodium channels of heart muscle different from nerve: similarly to frog nerve, the activation of sodium channels is shifted towards negative potentials, in contrast to nerve however, the inactivation is not abolished (Schmidt and Schmitt 1974; Mozhayeva et al. 1976) and seems to be affected only slightly at membrane potentials positive to −40 mV.

Methods

A detailed description of the method used has been published previously (Benndorf et al. 1985). In brief, isolated hearts from mice (albino mice, inbred strain Jena AB, either sex, 20 to 30 g body weight) were coronary perfused for 3 min with a solution A containing (in mmol/l): 150 NaCl; 5.4 KCl; 2.5 $CaCl_2$; 0.5 $MgSO_4$; 11.1 glucose; 5 HEPES, and subsequently for 15 min with a Ca-free solution B containing: 140 NaCl; 5.8 KCl; 0.5 KH_2PO_4; 0.4 Na_2HPO_4; 0.9 $MgSO_4$; 11.1 glucose; 5 HEPES; 0.1 mg/ml fatty acid free human serum albumine. The enzymatic dispersion of the heart was performed by recirculation with solution B which supplemented with 1 mg/ml collagenase (Sigma, type I). After a final perfusion with 52.5 NaCl; 4.8 KCl; 1.19 KH_2PO_4; 1.2 $MgSO_4$; 11.1 glucose; 145 sucrose; 10 HEPES; 1 mg/ml fatty acid free albumine (solution C) for 3 min, the soft hearts were cut and disaggregated into single cells by gently shaking. In all solutions the temperature was kept constant at 37 °C. pH was adjusted to 7.4 with NaOH. All solutions were gassed with 100 % O_2. Finally, the cells were incubated in solution C containing 2.5 $CaCl_2$. The remainders of surviving cells (about 10—20 %) were used for voltage clamping. All the experiments were carried out at room temperature 22 ± 1 °C. For intracellular dialysis of the cells the following solution was used (mmol/l): 150 KCl; 20 HEPES; 15 NaCl; 2 EGTA (pH adjusted to 7.1 with KOH). The cells were put into the experimental chamber (volume 1.2 ml) mounted on the stage of an inverted microscope.

For recording of the transmembrane currents a cell was sucked in the middle of its long side by a fire polished micropipette (tip diameter 4—8 μm). A polyethylene inlet was placed inside the pipette about 100 to 200 μm behind the tip for intracellular perfusion. The cell membrane was then disrupted by suction. To avoid intracellular Na loading of the cells during Aconitine treatment the cells were perfused intracellularly with solution as above. Currents were measured via Ag/AgCl electrodes with a virtual ground circuit between the chamber and ground. The current was partially fed to the feedback amplifier for the compensation of the series resistance. The linear leakage resistance was compensated in the usual way. The time constant of the linear capacitive current was in the range of 35 μs. The voltage clamp set-up used, the compensation of the series resistance, the effectiveness of intracellular perfusion as well as the characterization of the Na currents and problems arising from inhomogeneities of voltage control were described in detail by Benndorf et al. (1985).

Records were photographed from the screen of a storage oscilloscope OG 2-31 (Messelektronik Berlin). Currents were digitized by hand and numerically analysed with a microcomputer system MC 80 (Elektronik Gera). Aconitine (Fluka AG) was given to the chamber by a micropipette. The final concentration of Aconitine was 1 μmol/l in all experiments.

For the recording of action potentials small strips of the right mouse ventricle strips (length 3—4 mm, width 1 mm, thickness about 0.4 mm) were fixed between two clips in a chamber (volume 1 ml) and superfused with a solution containing (mmol/l): 150 NaCl; 5.4 KCl; 2.5 $CaCl_2$; 0.5 $MgSO_4$; 12 glucose; 5 HEPES (temperature 35 ± 1 °C, pH 7.4, solution gassed with 100 % O_2, superfusion rate 2 ml/min). Conventional microelectrodes (tip diameter <1 μm, resistance >5 MΩ) were used. Preparations were stimulated with a frequency of 1 Hz via a double barrelled metal electrode. Action potentials were differentiated with time constants of 10 μs.

The kinetics of the sodium currents were described by fitting the expression

$$I = A \cdot (1 - e^{-t/\tau_m})^3 \cdot e^{-t/\tau_h} \tag{1}$$

to individual records using the Levenberg-Marquard algorithm (Brown and Dennis 1972). τ_m, τ_h are the time constants of activation and inactivation, respectively. A means the product of

$$A = \bar{g}_{Na} \cdot m_\infty^3(U) \cdot h_\infty(U_h) \cdot (U - U_{rev}) \tag{2}$$

where $\bar{g}_{Na}$, m_∞, h_∞, U, U_h and U_{rev} stand for the maximum Na conductance, the steady state activation at the clamp potential U, the steady state inactivation at the holding potential U_h and the reversal potential, respectively. In most cases, especially after the application of Aconitine, U_{rev} was estimated from linear extrapolation of the current-voltage relationship to zero current. The h_∞-curve was obtained from conventional double pulse experiments using 500 ms lasting prepulse to reach a steady state. To calculate m_∞ from each individual current with known h_∞, the relationship

$$m_\infty \cdot \bar{g}_{Na}^{1/3} = \left(\frac{I_{peak}}{h_\infty(U_h) e^{-t_{peak}/\tau_h} \cdot (U - U_{rev})} \right)^{1/3} \cdot (1 - e^{-t_{peak}/\tau_m})^{-1} \tag{3}$$

was used and normalized to maximum values (t_{peak} means the time to the peak inward current I_{peak}). τ_m and τ_h were obtained from best fits of the measured currents by the use of equation (1). The voltage dependence of the steady state activation and inactivation was described by

$$m_\infty(U) = 1/(1 + e^{-(U - U_{s,m})/s_m}) \tag{4}$$

$$h_\infty(U) = 1/(1 + e^{(U - U_{s,h})/s_h}) \tag{5}$$

respectively. $U_{s,m}$ und $U_{s,h}$ mean the membrane potential at half maximum activation or inactivation. s_m and s_h denote the slope parameter of m_∞ and h_∞ (in mV), respectively.

To measure maximum inward currents, the outward part of the current measured at the end of each clamp step was subtracted from the peak inward current. In Tables 1 and 2, mean values ± S.D. are given. Student's t-test was used to compare voltage values.

Table 1. Effects of Aconitine (1 μmol/l) on action potentials of the mouse ventricular myocardium. The data for Aconitine modified action potentials were taken immediately before the preparations beated spontaneously.

		control	Aconitine
T_{50}	[ms]	8.9± 1.0	14.7± 1.9×
T_{75}	[ms]	31.5± 2.9	42.4± 6.5×
T_{90}	[ms]	52.4± 3.7	217.0± 12.5××
U_{ov}	[mV]	+32.5± 9.8	27.7± 10.2
U_r	[mV]	−82.5± 2.1	−81.1± 3.2
$\dot{U}_{max}$	[mV]	205.0± 27.0	134.0± 39.0×
		n=17	n=12

(T_{50}, T_{75}, T_{90}: duration of the action potential at 50, 75, 90 % repolarization, respectively. U_r: resting membrane potential, U_{ov}: maximum overshoot potential, $\dot{U}_{max}$: maximum upstroke velocity, n: number of measurements).
Pacing frequency 1 Hz, microelectrode measurements from right ventricular strips, × $P<0.05$, ×× $P<0.01$.

Table 2. Properties of Aconitine-modified Na channels in single cells of mouse ventricle (n: number of cells studied) (1 μmol/l Aconitine).

		control	n	Aconitine	n
I_{max}	[nA]	28.0± 9.0	15	14.0± 6.0	10×
U_{rev}	[mV]	16.0± 11.0	10	−8.0± 6.0	19×
$U_{s,m}$	[mV]	−38.0± 4.0	4	−69.0± 7.0	4××
s_m	[mV]	7.2± 2.1	4	7.1± 1.9	4
$U_{s,h}$	[mV]	−72.0± 11.0	9	−85.0± 11.0	10×
s_h	[mV]	9.1± 2.2	9	15.6± 4.4	10××

(I_{max}: maximum inward current, U_{rev}: reversal potential, $U_{s,m}$: potential at half maximum activation, s_m: slope parameter of activation, $U_{s,h}$: potential at half maximum inactivation, s_h: slope parameter of inactivation).
×× $P<0.01$, × $P<0.05$ Student's t-test.

Results

Two groups of results will be presented: (i) effects of Aconitine on action potentials in multifibre preparations, (ii) properties of Aconitine-modified Na-currents in isolated ventricular cells.

Aconitine-induced changes in action potential

The changes in the configuration of action potentials of right ventricular preparations are shown in Figure 1. Under control conditions the action potential of the mouse ventricular myocardium is characterized by a fast upstroke followed by fast initial repolarization changing over into a plateau-like phase at about −40 mV.

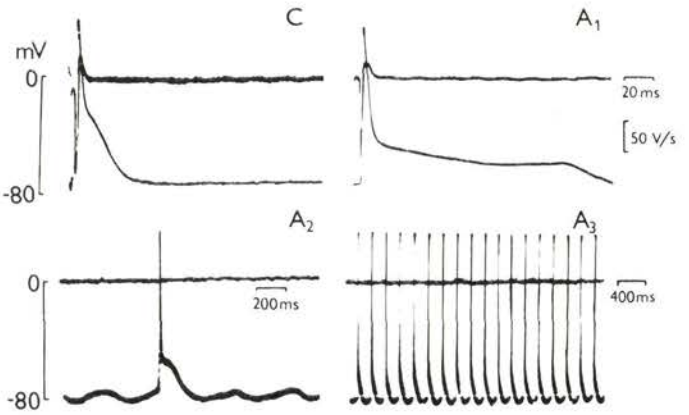

Fig. 1. Effects of Aconitine on transmembrane potentials (mouse, right ventricular strips, microelectrode recordings). C: control, A1: 2 min, A2: 6 min after the addition of Aconitine to the bath (final concentration 1 μmol/l). Note the appearance of oscillatory afterdepolarizations immediately before the preparations became spontaneous (A3). The first time derivative is arbitrarily shifted to zero potential in all traces. Aconitine reduced the maximum upstroke velocity of action potentials.

Aconitine prolonged the terminal repolarization up to more than 200 ms. The resting potential was not changed by Aconitine in the prearrhythmic state. Diastolic oscillatory afterdepolarizations ensued in five from seven Aconitine-treated preparations, ultimately leading to a spontaneous activity (frequencies between 5 and 6 Hz). The results obtained from multifibre preparations are summarized in Table 1.

Effect of Aconitine on the current-voltage relationship

Figure 2 (left) shows sodium currents obtained from the same cell before and 5 min after the addition of Aconitine to the external bathing solution (final concentration 1 μmol/l). From a holding potential of −90 mV, test pulses to −30 mV with a duration of 7 ms were applied at a frequency of 1/s. After the addition of Aconitine into the bath an inwardly directed "leakage" current had to be compensated of up to 25 % of the peak sodium inward currents.

Several minutes after the application of Aconitine, fast TTX sensitive inward

currents could be obtained by a depolarization from the holding potential of −90 mV as low as 10 mV instead of a depolarization to −50 mV in the controls. The just suprathreshold currents were extremely prolonged, whereas currents elicited at more positive potentials did not show a striking delay of inactivation. The peak current-voltage relation is illustrated in Figure 2 (right) and shows a decrease in the peak current concomitant with an Aconitine-induced shift of both the reversal potential and threshold potential towards more negative potentials (also see Table 2).

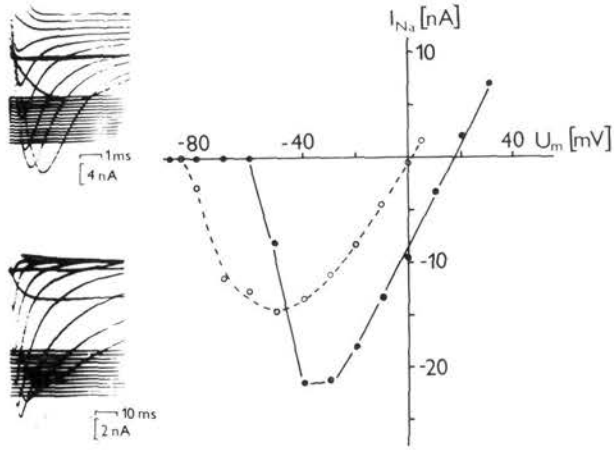

Fig. 2. Current-voltage relationships of the peak sodium inward current of enzymatically isolated cells of the mouse ventricle. *Left:* Na inward currents before (top) and 25 min after the application of 1 μmol/l Aconitine (bottom). Holding potential −90 mV, depolarizing steps spaced 10 mV. Note the different time calibrations. The traces were obtained from the same cell. *Right:* Current-voltage relationships obtained from the currents shown on the left. Control: ● C, Aconitine: ○ A.

Aconitine-modified sodium channel kinetics

Figure 3 shows the effects of 1 μmol/l Aconitine on the time constants of activation and inactivation in four cells. At membrane potentials negative to −40 mV the inward currents were inactivated very slowly. Time constants of inactivation up to 500 ms, or non-inactivating currents were recorded. Such extremely slowly inactivating currents could be elicited near the normal resting potentials of the myocardial cells at holding potentials between −110 and −80 mV.

At potentials positive to −40 mV the inactivation was complete and fast (Fig. 3. left). In the range of membrane potentials negative to −40 mV the activation was slow (Fig. 3, right) whereas at more positive potentials, no signifi-

cant differences could be observed in the kinetics between control and Aconitine-modified currents.

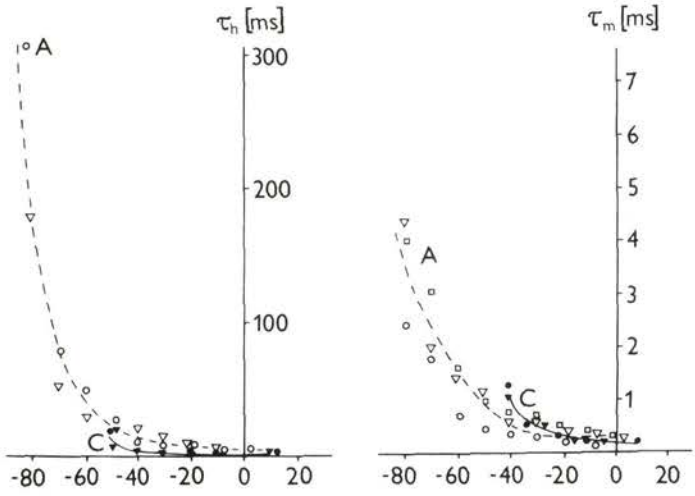

Fig. 3. Voltage dependence of Na-current inactivation and activation time constants of isolated ventricular cells of mice. *Left:* voltage dependence of the inactivation time constant (τ_h). *Right:* voltage dependence of the activation time constant (τ_m) . τ_h and τ_m were obtained from best fits of the currents using equation (1). Closed symbols represent control measurements (C), open symbols measurements under Aconitine (A). Each symbol represents a different cell in the same experiment. The smooth lines were drawn by eye. The holding potential was kept at −90 mV for all cells. The Aconitine traces were taken 20 to 45 min after the application of Aconitine.

Aconitine-modified steady state inactivation and activation

Aconitine, although slowing down inactivation at negative membrane potentials, does not abolish it in heart muscle. Figure 4 shows a steady state inactivation curve determined with a two-pulse protocol: a 500 ms prepulse at variable potentials preceded a 10 ms test step to −30 mV. The same protocol was used before and 15 min after the application of Aconitine (1 μmol/l). The potential of half maximum inactivation was shifted by 20.3 mV towards more negative potentials and the slope of the h_∞-curve was significantly decreased.

The steady state activation m_∞ was calculated from the peak currents according to formula (3). Figure 5 gives an example of an Aconitine-induced 32 mV shift of m_∞ towards more negative potentials. The slope of the activation curve remained unchanged.

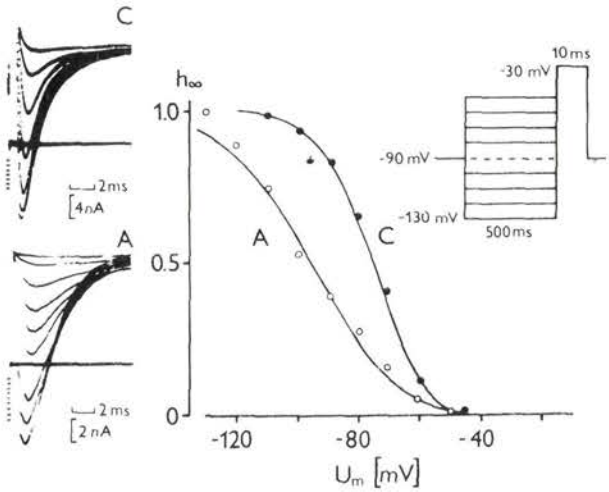

Fig. 4. Aconitine-induced changes in the voltage-dependence of steady state inactivation h_∞ of Na current in isolated ventricular cells of mice. The steady state inactivation was measured in a double pulse protocol shown in the inset. Inward currents activated by a test step to −30 mV are shown on the left. The holding potential was −90 mV for all traces. C: control, A: Aconitine. The prepulses were spaced 10 mV. The steady state inactivation is plotted against the membrane potential U_m on the right (● control, ○ Aconitine). The smooth curves were calculated by $h_\infty = 1/(1+\exp(U - U_{s,h})/s_h)$. From best fits the following parameters were obtained: control: $U_{s,h} = -74.6$ mV, $s_h = 9.8$ mV; Aconitine: $U_{s,h} = -94.9$ mV, $s_h = 13.4$ mV.

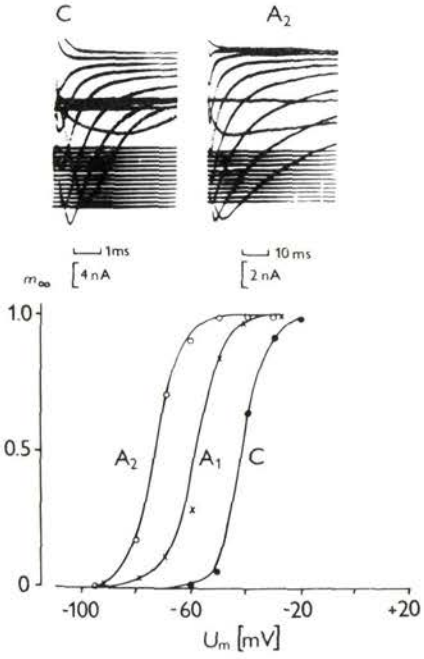

Fig. 5. Effects of Aconitine on the steady state activation of Na currents in isolated ventricular cells of mice. m_∞ was calculated by normalizing the left-hand terms of equation (3). The plot of m_∞ versus voltage was fitted by $m_\infty = 1/(1+\exp(-(U_m - U_{s,m})/s_m))$. *Top:* families of inward currents activated from a holding potential of −90 mV. C: control, A1: 15 min, A2: 40 min after the application of Aconitine (1 μmol/l). Note different time and current calibrations. The clamp steps were spaced 10 mV. *Bottom:* m_∞ — voltage relationship. The parameters obtained from the fits were: C: $U_{s,m} = -40.7$ mV, $s_m = 6.1$ mV, A1: $U_{s,m} = -59.0$ mV, $s_m = 6.0$; A2: $U_{s,m} = -72.7$ mV, $s_m = 6.0$ mV.

Table 2 summarizes all the results obtained with Aconitine-modified Na currents. The records analyzed in Table 2 were taken from cells voltage clamped for 5 to 45 minutes after the addition of Aconitine the bath.

Discussion

The effects of Aconitine on the kinetics of heart sodium channels are similar to those reported for nerve (Mozhayeva et al. 1976, 1980), skeletal muscle (Campbell 1982) and frog heart (Jurevichius and Rosenstraukh 1982) channels. Aconitine shifts the activation of sodium channels towards more negative membrane potentials in single heart muscle cells as well. The most surprising difference to Aconitine-modified Na channels in frog nerve (Schmidt and Schmitt 1974; Mozhayeva et al. 1976, 1980) is the complete and fast inactivation of the heart cell channels at membrane potentials positive to −40 mV (Fig. 2, 3, 5). However, at membrane potentials negative to −40 mV the inactivation is extremely prolonged.

Since the kinetics of inactivation was nearly unchanged at membrane potentials positive to −40 mV (Fig. 3 left), it is unlikely that activation of outward currents can substantially mask a non − or incompletely inactivated large inward current. It seems likely that this represents an actual difference between sodium channels in nerve and heart muscle, rather than an artefact due to the overlapping of outward currents, or a poor voltage control. A similar difference has been described for scorpion toxin in frog: the inactivation in nerve was incomplete over the whole voltage range (Koppenhöfer and Schmidt 1968a, b) whereas in frog skeletal muscle, it was slowed but complete (Catterall 1979). Similar observations were reported for Aconitine in nerve (Mozhayeva et al. 1976) and skeletal muscle (Campbell 1982). Complete inactivation after Aconitine has also been reported in frog multifibre preparations (Jurevichius and Rosenstraukh 1982).

We found a significant decrease of the maximum upstroke velocity of the action potential (Fig. 1, Table 1). Peak currents also decreased after the application of Aconitine (Fig. 2). Taking into account a constant intracellular Na concentration of the cells due to internal dialysis, the concomitant shift of the reversal potential U_{rev} (Fig. 2, Table 2) can be interpreted as being due to a decreased selectivity of the Aconitine-modified channels.

The ratio of sodium to potassium permeability, P_{Na}/P_K of the open channel can be calculated based on the constant field theory, and it provides a measure of the selectivity of the channel,

$$P_{Na}/P_K = \alpha = K_i\, e^{U_{rev}F/RT}/(Na_e - Na_i\, e^{U_{rev}F/RT})$$

with K_i, Na_i, Na_e describing the intra- and extracellular potassium and sodium activities, respectively ($P_k \times K_e$ has been neglected). The measured 24 mV shift of

U_{rev} (Table 2) would correspond to an about 20 fold decrease of α. This decreased selectivity of Aconitine-modified Na channels in heart muscle smoothly agrees with previous findings in nerve and skeletal muscle (Mozhayeva et al. 1976, 1977, 1980; Campbell 1978, 1982).

Aconitine-induced shifts of the activation curves of sodium channels towards negative membrane potentials were also observed in nerve and muscle (Herzog et al. 1964; Schmidt and Schmitt 1974; Mozhayeva et al. 1976, 1977; Campbell 1982). However, flattening of h_∞ (Fig. 4, Table 2) has not yet been described. It can be argued that, especially in the muscle preparations, the use of prepulses shorter than 200 ms may mask changes in the slope of h_∞ (see Figure 3 in Cambell 1982). The leftward shift of h_∞ was smaller than that of m_∞. The effects of Aconitine on both m_∞ and h_∞ resulted in an increased overlap of the curves ("window").

The existence of an increased window in the range between −100 and −60 mV would explain the Aconitine induced: (i) delay in the terminal repolarization of the action potentials (Fig. 1), (ii) increase of a "leakage" current and (iii) an incomplete inactivation of Na-current at negative membrane potentials. In non-perfused cells, a sodium "window" current would result in Na loading of the cells. Na overload could explain the possible inotropic effect of Aconitine (Honerjäger and Meissner 1983) similar to that of numerous cardioactive substances that prolong the open state of sodium channels (Honerjäger 1982; Katzung 1982). Na overload via Na/Ca exchange would result in Ca loading of the myocardial cell (for a review, see Honerjäger 1982; Langer 1982).

The oscillatory afterdepolarizations of the membrane potential (Fig. 1, also see Matsuda et al. 1959; Schmidt 1960; Goto et al. 1963, 1967; Jurevichius and Rosenstraukh 1982; Honerjäger 1982) might be effected by sodium mediated Ca overload, inducing an oscillating transient inward current, oscillatory Ca release, triggered activity, etc. (Kass and Tsien 1982; Karaguenzian and Katzung 1982; Clusin et al. 1982; Matsuda et al. 1982). However, it cannot be decided from the present experiments whether Aconitine alters the naturally occurring channels, or whether it induces a new population of kinetically modified channels, or whether it demasks channels similar to the threshold channel described in squid axon (Gilly and Armstrong 1984a, b).

References

Benndorf K., Boldt W., Nilius B. (1985): Sodium currents in single myocardial mouse cells. Pflügers Arch. **404**, 190—196

Brown K. M., Dennis J. E. Jr. (1972): Derivative free analogues of the Levenberg-Marquard and Gauss algorithms for non linear least squares approximation. Numer. Math. **18**, 289—297

Campbell D. T. (1978): Aconitine alters selectivity and kinetics of frog muscle sodium channels. Biophys. J. **21**, 42a

Campbell D. T. (1982): Modified kinetics and selectivity of sodium channels in frog skeletal muscle fibers treated with Aconitine. J. Gen. Physiol. **80**, 713—731

Catterall W. A. (1979): Binding of scorpion toxin to receptor sites associated with sodium channels in frog muscle. J. Gen. Physiol. **74**, 375—391

Clusin W. T., Bristow M. R., Karaguenzian H. S., Katzung B. G., Schroeder J. S. (1982): Do Ca-dependent ionic currents mediate ischemic ventricular fibrillation? Amer. J. Cardiol. **49**, 606—612

Gilly W. F., Armstrong C. M. (1984a): Threshold channels in the squid axon. Biophys. J. **45**, 11a

Gilly W. F., Armstrong C. M. (1984b): Threshold channel — a novel type of sodium channels in squid giant axon. Nature **309,** 448—450

Goto M., Tamai T., Yanaga T. (1963): Studies on the appearance and termination of Aconitine-induced atrial fibrillation with microelectrodes. Jpn. J. Physiol. **13**, 196—207

Goto M., Sakamoto Y., Imanaga I. (1967): Aconitine-induced fibrillation of the different muscle tissues of the heart and the action of acetylcholine. In: Electrophysiology and Ultrastructure of the Heart, (Eds. T. Sano, V. Mizuhira, K. Matsuda), pp. 199—209 Grune and Stratton, Inc., New York—London

Heistracher P., Pillat B. (1962): Elektrophysiologische Untersuchungen über die Wirkung von Chinidin auf die Aconitinvergiftung von Herzmuskelfasern. Naunyn-Schmied. Arch. Exp. Path. Pharmacol. **244,** 48—62

Herzog W. H., Feibel R. M., Bryant S. H. (1964): The effect of Aconitine on the giant axon of the squid. J. Gen. Physiol. **47**, 719—733

Honerjäger P. (1982): Cardioactive substances that prolong the open state of sodium channels. Rev. Physiol. Biochem. Pharmacol. **92**, 1—74

Honerjäger P., Meissner A. (1983): The positive inotropic effect of Aconitine. Naunyn-Schmied. Arch. Pharmacol. **322,** 49—58

Jurevichius J. A., Rosenstraukh L. V. (1982): Mechanism of Delayed Afterdepolarization Caused by Aconitine. In: Advances in Myocardiology, Vol. 3, pp. 159—176, (Ed. E. Chazov, V. Smirnov, N. S. Dhalla). Plenum Med. Book Comp. New York—London

Karaguenzian H. S., Katzung B. G. (1982): Voltage-clamp studies of transient inward current and mechanical oscillations induced by ouabain in ferret papillary muscle. J. Physiol. (London) **327,** 255—271

Kass R. S., Tsien R. W. (1982): Fluctuations in membrane current driven by intracellular calcium in cardiac Purkinje fibres. Biophys. J. **36**, 259—269

Katzung B. G. (1982): Myocardial toxicity as a result of altered membrane channel function. In: Cardiovascular Toxicology, (Ed. E. W. Van Stee) pp. 135—179. Raven Press, New York

Koppenhöfer E., Schmidt H. (1968a): Die Wirkung von Skorpiongift auf die Ionenströme des Ranvier'schen Schnürringes. I. Die Permeabilitäten P_{Na} und P_K. Pflügers Arch. **303,** 133—149

Koppenhöfer E., Schmidt H. (1968b): Die Wirkung von Skorpiongift auf die Ionenströme des Ranvier'schen Schnürringes. II. Unvollständige Natrium-Inaktivierung. Pflügers Arch. **303,** 150—161

Langer G. A. (1982): Sodium-calcium exchange in the heart. Annu. Rev. Physiol. **44**, 435—449

Matsuda K., Hoshi T., Kameyama S. (1959): Effects of Aconitine on the cardiac membrane potential of the dog. Jpn. J. Physiol. **9**, 419—429

Matsuda H., Noma A., Kurachi Y., Irisawa H. (1982): Transient depolarization and spontaneous voltage fluctuations in isolated single cells from guinea pig ventricles. Circ. Res. **51**, 142—151

Mozhayeva G. N., Naumov A. P., Negulyaev Yu. A. (1976): Effect of Aconitine on some properties of sodium channels in the Ranvier node membrane. Neirofiziologiya **8**, 152—160 (in Russian)

Mozhayeva G. N., Naumov A. P., Negulyaev Yu. A., Nosyreva E. D. (1977): The permeability of

Aconitine-modified sodium channels to univalent cations in myelinated nerve. Biochim. Biophys. Acta **466,** 461—473

Mozhayeva G. N., Naumov A. P., Nosyreva E. D. (1980): Some features of kinetic and steady-state characteristics of Aconitine-modified sodium channels. Neirofiziologiya **12**, 612—618 (in Russian)

Peper K., Trautwein W. (1967): The effects of Aconitine on the membrane current in cardiac muscle. Pflügers Arch. **296,** 328—336

Scherf D., Schott A. (1973): Extrasystoles and Allied Arrhythmias. Year Book Publ., Chicago

Schmidt R. F. (1960): Versuche mit Aconitin zum Problem der spontanen Erregungsbildung im Herzen. Pflügers Arch. **271,** 526—536

Schmidt H., Schmitt O. (1974): Effect of Aconitine on the sodium permeability of the node of Ranvier. Pflügers Arch. **349,** 133—148

Received May 12, 1985/Accepted March 4, 1986