present experiments $PGF_{2\alpha}$ produced a dose-dependent increase of the contractions upon intermittent stimulation as well as an increase of the ACh output in response to continuous stimulation. The fact that atropine abolished the response to electrical stimulation shows that the contractile response was mediated by acetylcholine, and the demonstration that $PGF_{2\alpha}$ increased the acetylcholine liberation indicates that its effect was central to the neuromuscular junction, most probably on the postganglionic neurone.

Table. Guinea-pig ileum. ACh output in pg/g/min in response to continuous electrical stimulation (5 Hz, 0.4 msec, submaximal current, 3 min) before and after $PGF_{2\alpha}$ 1 nM

Segments	1	2	3	4	5
Before $PGF_{2\alpha}$	3.35	3.00	4.92	3.26	7.4
After $PGF_{2\alpha}$	5.74	4.90	6.80	3.89	13.0

Modulation of the ACh release[1-3,5] nonspecific sensitization of the smooth-muscle membrane to ACh[4] has been proposed for explanation of the mechanism of action of PGsE in the guinea-pig ileum. The present data show an effect of $PGF_{2\alpha}$ on the cholinergic system too and suggest an action of $PGF_{2\alpha}$ central to the neuromuscular junction. The latter suggestion was supported by the finding that $PGF_{2\alpha}$ counteracted the effects of drugs which inhibited the postganglionic ACh release - indomethacin[1, 2] and metenkephalin[12]. Thus similar to the effects of PGsE[10], the $PGF_{2\alpha}$ action on the contractile responses of the guinea-pig ileum might be attributed to an increased ACh release.

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Dantrolene: evidence for effects on Na permeability properties of the nodal membrane[1]

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Summary. The effects of dantrolene on myelinated frog nerve fibers were studied in voltage clamp experiments. Dantrolene shifted the potential-dependent parameters describing Na^+ permeability towards more negative membrane potentials. The findings are interpreted as a change in the negative surface charge of the membrane.

Dantrolene-Na (DaNa) is a muscle relaxant and has been used for the treatment of spasticity[2]. Furthermore an exacerbation of paramyotonic muscle weakness by DaNa has been observed[3]. It has been assumed that DaNa reduces contraction of skeletal muscle either by decreasing the amount of Ca^{++} released from the sarcoplasmic reticulum[4-6] and/or from some presynaptic Ca^{++} stores[7]. No direct effects on neuromuscular transmission[8] or on the electrical properties of the skeletal muscle membrane[9] have been detected. On the other hand it has recently been reported that DaNa affected frog myelinated nerve fibers and that it increased their excitability[3]. We confirmed these results by performing voltage clamp experiments. We describe effects of DaNa on the nodal membrane which we interprete as an influence of the drug on fixed negative surface charges of the nerve membrane.

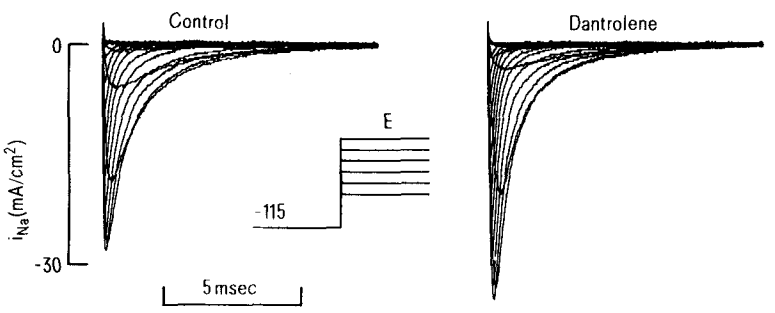

Figure 1. Membrane currents in Ringer (control) and in the presence of dantrolene-Na (saturated solution), recorded during depolarizing potential steps of increasing amplitude. Each pulse preceded by a 50-msec pulse of −115 mV. Corrected for leakage and capacity currents. K^+ currents were blocked by external TEA and internal CsCl.

Experiments were done on 8 single myelinated nerve fibers dissected from the N. tibialis of *Rana esculenta* at 20 °C. The fibers were voltage clamped with the method described by Nonner[10]. The Ringer solution was composed of 110 mM NaCl, 2.5 mM KCl, 2 mM $CaCl_2$, and 5 mM Tris-buffer, pH = 7.3. In 5 fibers the K currents were blocked by external tetraethylammoniumchloride (TEA, 10 mM) and internal CsCl (by diffusion through the cut internodes). Following the procedure of other authors[7,8] we used saturated solutions of DaNa. 10 µg/ml of the drug were added to the Ringer solution, stirred for 1 h and then filtered before use. The membrane potential was assumed to be −70 mV if $h_\infty = 0.7$ (Stämpfli[11]). Changes of the membrane potential were monitored during the experiment. For further details of the experimental setup see Bromm et al.[12].

Figure 1 shows a family of transient Na^+ currents elicited by depolarizing potential steps of increasing amplitude, each preceded by a 50-msec hyperpolarizing potential to −115 mV in order to reduce normal resting Na^+ inactivation. After switching to the test solution containing DaNa the effects developed slowly and became stable within 15 min. Compared to the controls the peak Na^+ currents at potentials $E < 0$ mV were increased. In figure 2 the peak Na^+ currents were plotted versus membrane potential. Besides the increase in amplitude the steep part of the current-voltage relation in the presence of the drug was shifted towards more negative membrane potentials. The shift of the point at which the Na^+ current amplitude had 50% of its maximum was 7.0 ± 2.3 mV (mean ± SD, N = 5). The Na^+ equilibrium potential E_{Na} and the current-voltage relation for $E > 10$ mV were unaffected. In correspondence to this the resting membrane potential remained unchanged.

The membrane current traces were analyzed by a least squares fit procedure using the equation[13,14]

$$P_{Na} = \bar{P}_{Na} \cdot [m_\infty \cdot (1 - \exp(-t/\tau_m))]^b \cdot [\exp(-t/\tau_h)] \qquad (1)$$

where $\bar{P}_{Na}$ denote the maximum Na^+ permeability, m_∞ the steady state activation parameter, and τ_m and τ_h the time constants of activation and inactivation of P_{Na} respectively. Best fits were achieved with an exponent $b = 4$ (see also Ochs et al.[14]). In addition $h_\infty(E)$, the steady state inactivation parameter of P_{Na}, was determined by the standard 2-pulse procedure[15]. We found that in the presence of DaNa the curves relating τ_m and τ_h as well as those of the steady state parameters m_∞ and h_∞ to membrane potential were shifted towards more negative membrane potentials, compared to those measured in Ringer solution. The points for 50% activation (m_∞) and inactivation (h_∞) were shifted by 4.0 ± 1.0 mV and 5.2 ± 1.6 mV (mean ± SD) respectively. The points at $E = 0$ mV of the curves ralting τ_m and τ_h to membrane potential in Ringer solution where shifted by 7.5 ± 3.5 mV and 16.6 ± 6.1 mV respectively. $\bar{P}_{Na}$ was not influenced by the drug. The parameters describing the K^+ permeability[13] remained unchanged; especially, we observed no shifts after addition of the drug to the test solution (measurements in 4 fibers).

The results show that DaNa affects properties of the excitable nerve membrane. The main effect was a shift of the parameters defining P_{Na} in eq. 1 towards more negative membrane potentials. These findings support recent observations in the same preparation, but obtained with a different method[3]. Similar shifts of the potential-dependent membrane parameters towards more negative membrane potentials were observed with decreased Ca^{++} concentrations in the external solution and were explained by an increase in negative fixed charges at the outer membrane surface[16-18]. The shifts observed with DaNa may as well be interpreted in terms of a change of the electric field within the nodal membrane induced by the drug. However, on the basis of our results we cannot decide whether DaNa exerts its effects on the external or axoplasmic side of the membrane, or whether it affects e.g. the uptake or release mechanisms of intracellular Ca^{++} storage sites.

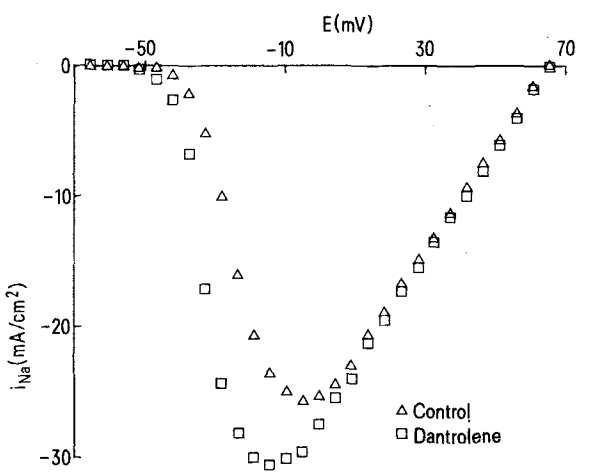

Figure 2. Evaluation of membrane currents shown in fig. 1. Peak Na^+ currents plotted versus membrane potential E in Ringer (△) and in Ringer + dantrolene-Na (□).

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