Toxins of a Blue-Green Alga: Similarity to Saxitoxin

Abstract. *Toxins were isolated from the freshwater blue-green alga* Aphanizomenon flos-aquae. *The toxic fractions were characterized by paper and thin-layer chromatography, isolation characteristics, infrared spectra, physiological activity, and reactivity with specific color reagents. The toxic fractions appear to be similar, if not identical, to saxitoxin (paralytic shellfish toxin), which is produced by the marine dinoflagellate* Gonyaulax catenella.

The blue-green alga *Aphanizomenon flos-aquae* possesses a toxin with electrophysiological properties similar to those of saxitoxin (paralytic shellfish toxin) which block action potentials in desheathed frog axons without producing depolarization (*1*).

In purifying the active factor, we found three related toxic compounds similar to purified saxitoxin. The similarity of toxins from two unrelated organisms, *Aphanizomenon flos-aquae* and *Gonyaulax catenella*, led us to compare chemical, chromatographic, and infrared absorption characteristics of these compounds.

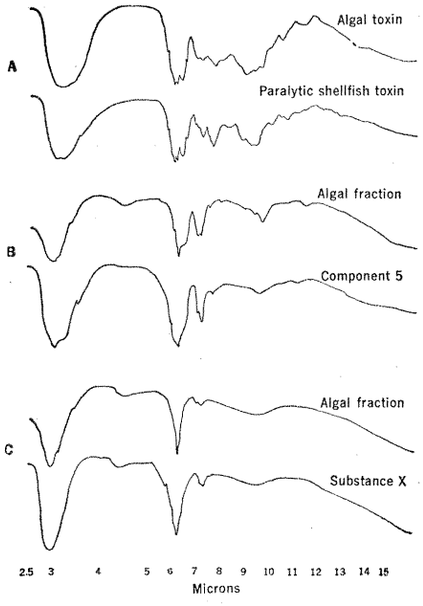

Fig. 1. Infrared spectra of the major fractions of blue-green algal toxin and paralytic shellfish toxin. (A) The most toxic fraction from paper chromatography, R_F .32. (B) Blue-green algal fraction from alumina column compared to shellfish toxin fraction 5, redrawn from Casselman *et al.* (*4*). (C) Toxic blue-green algal fraction, R_F .57, compared to compound X from shellfish toxin, redrawn from Casselman *et al.* (*4*).

The blue-green alga was grown and harvested by the procedure of Gentile and Maloney (*2*). Freeze-dried cells were resuspended with 0.01*N* HCl as a 1 percent weight to volume mixture. The cells were completely disrupted by sonic treatment for 5 minutes. The slurry was centrifuged, and the residue was discarded. The supernatant was treated with 1.0*N* sodium hydroxide to raise the *p*H to 5.5, and the resulting precipitate was removed by centrifugation. The partially purified extract was then either lyophilized or purified by the method which Schantz *et al.* used to purify *Gonyaulax* toxin (*3*).

Another method for purifying the toxic components consisted of chromatographing the partially purified, lyophilized material on Whatman 3MM paper with an isopropyl alcohol, acetic acid, and water system (40:1:30). The fraction containing the toxins had an R_F value of 0.8 to 0.85 and was observed as a yellow band. This was eluted with 0.01*N* HCl and lyophilized to dryness. A 500-mg sample of dried material was suspended in a mixture of isopropyl alcohol, acetic acid, and water (60:1:10), and placed on a silica-gel column (2 by 30 cm). Fractional elution with the above solvent mixture yielded three fractions of different toxicity. The most active fraction, upon lyophilization, was a white or slightly yellow hygroscopic glasslike material which could not be crystallized.

When chromatographed on paper with a *t*-butyl alcohol, water, and acetic acid system (2 : 1 : 1), the material yielded three Weber reagent positive spots similar to the pattern found (*4*) with saxitoxin (Table 1).

Thin-layer chromatography with commercially prepared silica gel plates and a *t*-butyl alcohol, water, and acetic acid system (2 : 1 : 1) as the developer, also showed Weber reagent positive spots with R_F values identical to that of saxitoxin. The R_F values obtained with thin-layer chromatography vary somewhat with the amount of material applied. Generally, the most toxic material appeared between R_F 0.39 and 0.46, as did saxitoxin.

The infrared spectra of the toxin fractions eluted from the alumina column or from paper chromatograms were similar to those obtained from partially purified saxitoxin (Fig. 1). The lack of identity of the spectra may result from traces of impurities or minor structural differences in the molecule. When the most toxic fraction was chromatographed again the absorption at 8.0 and 11.4 μ was less, indicating that further purification might produce an even greater similarity.

Table 1. Paper chromatography of partially purified toxins. The R_F values obtained on washed Whatman 1 paper using a *t*-butanol, acetic acid, and water system, (2 : 1 : 1) as the developer. W, Weber reagent positive; N, ninhydrin positive, purple; NY, ninhydrin positive, yellow; B, basic with brom cresol green; T, toxic; J, Jaffe reagent positive. Saxitoxin was chromatographed simultaneously with blue-green algal toxin. The fraction with an R_F value of 0.32 is the most toxic in each case.

Blue-green algal toxin (R_F)	Saxitoxin (R_F)	Reported for saxitoxin (*4*) (R_F)
.32 T,W,NY,B,J	.32 T,W,NY,B,J	.32 T,W,NY,B
.43 N	.43 N	
.57 T,W,NY,B		.53 T,W,B
.83 W,N,T		

Both saxitoxin and the blue-green algal toxins are soluble in water and methanol and less soluble in ethanol. The toxins are not soluble in acetone, ether, or chloroform. They are stable in hot acid solution of *p*H 2 to 4, but become more labile with increasing *p*H. Neither poison shows any visible or ultraviolet absorption above 210 nm. Both toxins are stable for at least 2 hours in homogenates of fish liver at *p*H 7.0. Like saxitoxin, the blue-green algal toxins are dialyzable, resistant to crystallization, and very hygroscopic, and they decompose without melting.

The most potent algal toxin fraction will kill *Fundulus heteroclitus* (killifish) in intraperitoneal dosage equivalent to 29 μg/kg. Reactions of mice to algal toxins are identical to those produced by shellfish toxin, although the lethal dosage appears to be somewhat greater. When low doses of partially purified algal toxins are injected into fish, they induce darkening in the anterior portion of the fish; as the pigmentation spreads posteriorly, loss of lateral equilibrium and death occur within minutes. The most toxic fraction (R_F .32 on paper chromatography) even at low doses kills without the darkening effect. The fish recover rapidly from sublethal doses of the toxin; this is also true for saxitoxin.

The similarities of these algal toxins indicate that they may be identical or slightly modified products stemming from similar metabolic pathways.

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References and Notes

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3. E. J. Schantz, J. M. Lynch, G. Voyada, K. Matsumoto, H. Rapport, *Biochemistry* **5**, 1191 (1966).
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5. We want to thank B. Pringle for encouragement and information on paralytic shellfish poison.

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Adrenaline: Mechanism of Action on the Pacemaker Potential in Cardiac Purkinje Fibers

Abstract. *The pacemaker potential in Purkinje fibers is generated by a slow fall in potassium current which allows the inward background currents to depolarize the membrane. Adrenaline shifts the relation between activation of the potassium current and membrane potential in a depolarizing direction. Consequently, during the pacemaker potential, the potassium current falls more rapidly to lower values and the inward currents then depolarize the membrane more quickly. The shift in the potassium activation curve produced by adrenaline is large compared to that produced by calcium ions. The molecular action of adrenaline may involve either a large change in the surface charge of the membrane or a change in the dependence of the potassium permeability on the local electric field.*

Several attempts have been made to determine how adrenaline accelerates the cardiac pacemaker, but most results have been negative. Adrenaline does not greatly influence the resting conductance or the sodium conductance involved in generating the action potential (*1*, *2*). However, the voltage-clamp technique has revealed the changes in time- and voltage-dependent current which underlie the pacemaker potential in Purkinje fibers (*3*–*5*). We have studied the action of adrenaline on these current changes.

The major time-dependent change in the pacemaker potential is a decrease in potassium current following repolarization at the end of the action potential (*3*–*6*). This fall in potassium current allows the inward background currents to depolarize the membrane to the threshold for initiating the action potential. Quantitative analysis of this potassium current (called i_{K_2} to distinguish it from i_{K_1}, which does not appear to be time-dependent) has shown that it is determined by a permeability variable, s, which is similar to the Hodgkin-Huxley permeability variables (*5*). Thus

$$i_{K_2} = s \cdot \overline{i_{K_2}} \qquad (1)$$

where $\overline{i_{K_2}}$ is the current when $s = 1$, and s obeys first-order kinetics:

$$ds/dt = \alpha_s(1 - s) - \beta_s s \qquad (2)$$

where α_s and β_s are functions of membrane potential (E) which have been determined empirically (*5*).

Adrenaline accelerates the pacemaker depolarization of spontaneously beating Purkinje fibers (*1*, *2*, *7*). To determine the mechanism of this action, we have studied the behavior of s during superfusion of a sheep Purkinje fiber with a Tyrode solution containing 5×10^{-7} g of adrenaline per milliliter (Fig. 1). The membrane potential was voltage-clamped at -80 mv, and the current changes following step depolarizations and hyperpolarizations were recorded. The current peak soon after return to -80 mv gives a measure of the degree of activation, s, since $\overline{i_{K_2}}$ is constant when E is constant. Equation 1 then becomes

$$i_{K_2} \propto s \qquad (3)$$

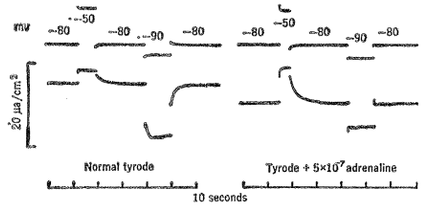

Fig. 1. Effect of adrenaline on voltage-clamp currents in sheep Purkinje fiber. (Left) Normal Tyrode solution. (Right) Tyrode solution containing 5×10^{-7} g of adrenaline per milliliter. Each record shows current (bottom) in response to 30 mv depolarization and 10 mv hyperpolarization from a holding potential of -80 mv. Note that in adrenaline solution no current is deactivated by hyperpolarization and that steady-state current at -80 mv is more negative than in absence of adrenaline. This change in steady-state current is attributable to shift in s_∞ (E) relation (see Fig. 2). Results shown in Fig. 2 were obtained by measuring current immediately following return to -80 mv after long-lasting depolarizations and hyperpolarizations.

Figure 2 shows the variation in the steady-state value of s (that is, s_∞) with membrane potential measured in terms of i_{K_2} at -80 mv. The major effect of adrenaline is to shift the curve in the depolarizing direction by about 30 mv. The total amplitude of the curve is also reduced, but it is not yet certain whether any significance can be attached to this effect. Pronethalol (10^{-6} g/ml) restores the curve to its original position. An intermediate concentration of pronethalol (5×10^{-7} g/ml) shifted the curve by about 15 mv.

Figure 3 shows measurements of the rate of change of potassium current as a function of the membrane potential, measured in terms of the reciprocal of the time constant of current change,

$$\tau_s^{-1} = \alpha_s + \beta_s \qquad (4)$$

The symbols are the same as in Fig. 2. Adrenaline also shifts this curve in the depolarizing direction, and at -90 mv, which corresponds to the beginning of the pacemaker potential, the change in current is much faster when adrenaline is present than when it is absent or when pronethalol is applied.

Thus, adrenaline has the same effect on the properties of i_{K_2} as a hyperpolarization. A simple way in which it might do this is to alter the local electric field controlling the s kinetics, perhaps by adding its own positive charge to the surface of the membrane. However, adrenaline might also act in some more indirect manner, for example, by chemically altering the state of the membrane so that the energy levels of the s reaction are changed. At present, we cannot decide between these and various other possibilities. It is worth noting, however, that the effect of adrenaline in shifting the relation between s_∞ and membrane potential is much greater

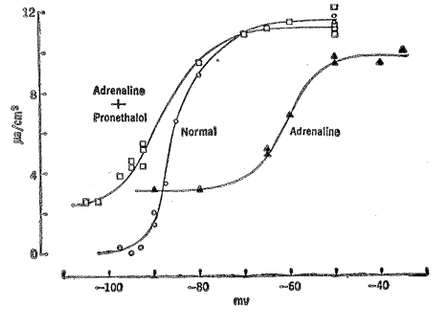

Fig. 2. Relations between steady-state degree of activation (s_∞) of slow K current and membrane potential, measured in terms of current immediately following return to -80 mv (see Fig. 1); ○, normal Tyrode; ▲, adrenaline (5×10^{-7} g/ml); and □, pronethalol (10^{-6} g/ml).