trifugation at 1000 × g for 10 min were diluted with acid citrate dextrose (ACD) buffer (1:1) and stored at 4 °C for not more than 24 h. Every 4 h, an aliquot was washed and suspended at a ratio of 1:15 (v/v) in a K^+-free saline solution containing 145 mM NaCl, 5 mM $MgCl_2$, 10 mM glucose and 5 mM Tris-HCl (pH 7.4). After temperature equilibration, 4 ml aliquots were exposed at 22 °C to absolute pressures ranging from 200–1000 b in a pressure device described elsewhere[16-20]. Briefly, the hyperbaric chamber consists of a steel cylinder with an inner plexiglass container. The chamber is pressure sealed by means of a steel screw cap. Particular care has been taken to minimize the temperature effects caused by the more or less adiabatic stepwise compression or decompression of the silicone fluid. By means of a pressure vessel specially designed to keep test tubes in an as large as possible water bath, a maximum temperature change of 2 °C was measured in the samples after applying a sudden pressure increase of 1000 b, reached in 30 sec. After decompression, the K^+-content of the erythrocytes and of the incubation medium was determined by flame photometry. The values for the K^+-efflux were corrected for the K^+-release observed in control experiments in which the cell suspensions were kept at 22 °C without applying pressure. The corrected net K^+-efflux should therefore be 0 at atmospheric pressure.

Results and discussion. Results from a typical experiment are shown in figure 1. The pressure induced net K^+-efflux from human erythrocytes increases almost linearly up to pressures of about 600–700 b (0.5 µmoles/ml erythrocytes/h). Above this pressure range a significant increase in the pressure-induced net K^+-efflux is observed. The pressure was applied in this experiment for 20 and 40 min, respectively. The K^+-effluxes have been observed to remain linear for at least 60 min regardless of the applied pressure. The observed permeability change is a reversible event, as shown by 3 consecutive cycles of compression (20 min at 1000 b followed then by 20 min at atmospheric pressure) (figure 2). The release of K^+ from the erythrocytes into the supernatant induced by each single pressure application is indeed the same for 3 consecutive compression steps.

The above results show that a 'narrow' pressure range exists, at which a pronounced increase in the membrane permeability occurs. Experiments conducted at atmospheric pressure by changing the temperature of the incubation medium have definitely allowed us to discard any possibility of a temperature-induced event related to the compression of the fluids. The results presented here thus seem to confirm the predictions of the electro-mechanical model formulated for plant and animal cell membranes. Although the experimental results are consistent with the predictions of the electro-mechanical model, at present other possible mechanisms cannot be excluded with certainty; this will be discussed in detail in a forthcoming paper.

1 Acknowledgments. This work was supported by a grant from the Sonderforschungsbereich 160 to U.Z. and by grant No. 2.4511.76 from the FRFC to R.G.
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Octopamine and proctolin mimic spontaneous membrane depolarisations in *Lucilia* larvae*

S.N. Irving[1] and T.A. Miller

Division of Toxicology and Physiology, Department of Entomology, University of California, Riverside (California 92521, USA), 5 July 1979

Summary. Octopamine and proctolin at concentrations below 10^{-8} M reversibly induce a spontaneous rhythmic depolarization which occurs in body-wall muscles of *Lucilia* larvae. The effect appears to be postsynaptic and mediated by receptors specific for each substance.

Body-wall muscles of cyclorrhaphan dipteran larvae are innervated by a single 'fast' and a single 'slow' axon[2,3]. However, a further type of response can be recorded from these muscles which is not associated with motor axon stimulation.

Materials and methods. Intracellular recording and nerve stimulation of body-wall muscles of *Lucilia sericata* larvae were as previously described[2,4]. Saline used was 172 mM NaCl, 13.3 mM KCl, 4 mM $CaCl_2$, 6 mM $MgCl_2$, 1.0 mM NaH_2PO_4, 1.2 mM $NaHCO_3$, 33 mM sucrose, pH 7.1. Calcium-free saline was prepared by omitting calcium and adjusting the overall osmolarity by increasing the sucrose concentration to 43 mM. D-L Octopamine hydrochloride, tetrodotoxin (Sigma Chemical Co.) and proctolin (Peninsula Labs Inc.) were added to the saline at the concentrations stated.

Results. In some muscles in a given preparation, spontaneous rhythmic membrane depolarizations (SR activity) were recorded (figure 1, A). This SR activity was not associated with neural stimulation, and usually lasted for at least 30 min, after which the depolarizations decayed to small oscillations of 1 mV amplitude. SR activity gradually ceased if the preparation was perfused with saline.

Reports of rhythmic activity in locust skeletal muscle[5] being inhibited by octopamine[6] and stimulated by proctolin[7,8] prompted us to examine the effects of these drugs on *Lucilia* body-wall muscles. When applied in saline to quiescent preparations, these drugs produced membrane depolarizations identical to spontaneous SR activity (figure 1, B and 2, A).

However, muscles from which proctolin elicited SR activity were not sensitive to octopamine and vice versa. Both proctolin and octopamine produced small membrane oscillations at concentrations below 10^{-10} M (figure 1, C) although the sensitivity varied from preparation to preparation. These small membrane oscillations sometimes increased to give SR activity (figure 1, D) although higher concentrations of 10^{-7}–10^{-8} M were usually required. At

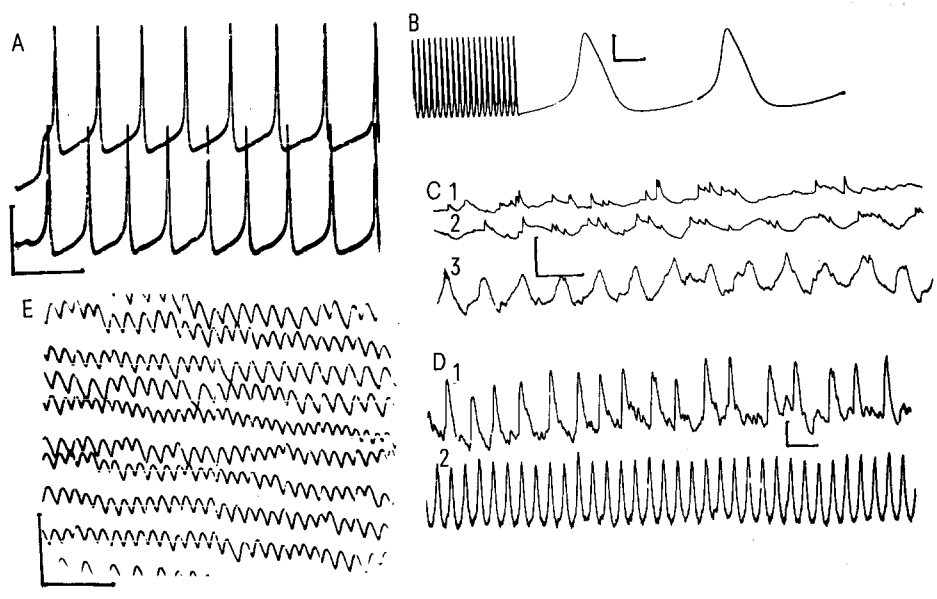

Fig. 1. *A* Spontaneous SR activity (2 sweeps of oscilloscope). Em = −42 mV. Calibration = 20 mV, 2 sec. *B* SR activity induced by 10^{-8} M proctolin. Em = −38 mV. Calibration = 10 mV, 2 sec. Last 2 potentials 10 mV, 80 msec. *C*, 1 Miniature EPSPs. 2 Same muscle 1 min after application of 10^{-10} M octopamine. 3 Same muscle 5 min after application of 10^{-10} M octopamine. Em = −42 mV. Calibration = 0.5 mV, 600 msec. *D* 1 and 2 Larger oscillations, with greater similarity to 1B, produced by 10^{-8} M octopamine in 2 separate muscles. Em(1) = −40 mV; Em(2) = −37 mV. Calibration = 0.5 mV, 600 msec. *E* Burst of 'slow' EPSPs produced upon application of 10^{-6} M octopamine (9 sweeps of oscilloscope). Calibration = 4 mV, 400 msec.

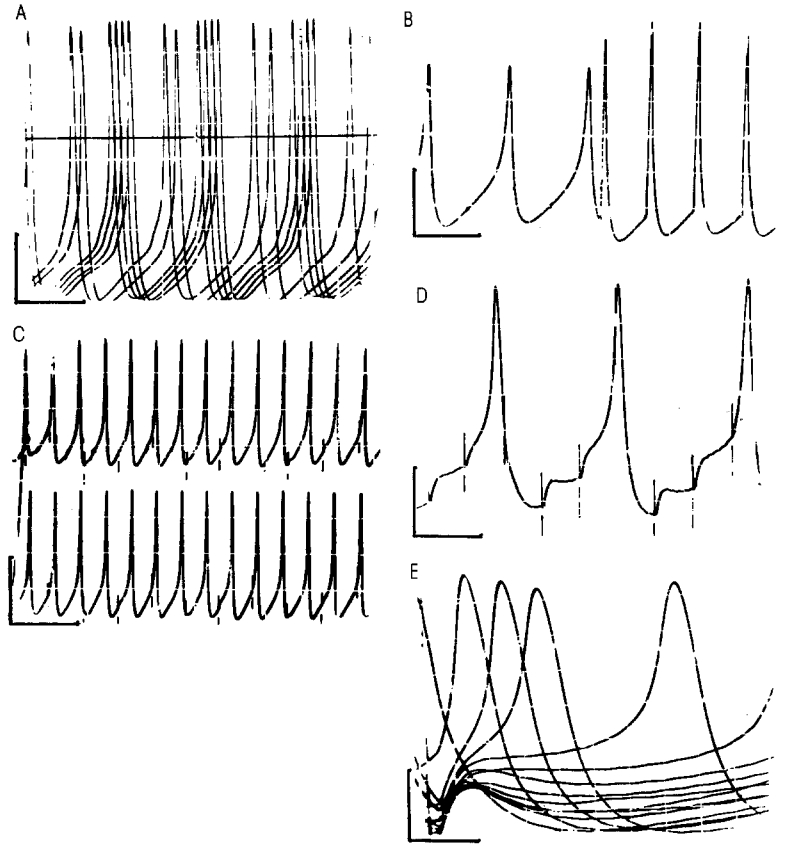

Fig. 2. *A* Octopamine-induced SR activity (6 sweeps of oscilloscope). Line indicates zero potential. Calibration = 10 mV, 400 msec. *B* Neurally evoked response (last 4 potentials) and octopamine-induced SR activity. Calibration = 10 mV, 400 msec. *C* Octopamine-induced SR activity at maximum response. Neural stimulation (seen as repeated stimulus artifact) does not produce an ESPS. Calibration = 20 mV, 1 sec. *D* and *E* Postsynaptic potential evoked by neural stimulation as SR activity wanes. The EPSP is triggered in 2. Calibration = *D* 10 mV, 200 msec. *E* 10 mV, 20 msec. Figures 2*B–E* recorded sequentially from the same muscle. Em = −40 mV.

concentrations between 10^{-4} and 10^{-6} M, spontaneous muscle contractions and spontaneous bursting of motor axons were often recorded (figure 1, E).

Proctolin- or octopamine-induced SR activity was not abolished by 10^{-5} M tetrodotoxin, which blocks axonal conduction in this preparation[4]. Perfusion of calcium-free saline abolished SR activity, which was restored when calcium was added to the saline. Induced SR activity sometimes overshot zero potential (figure 2, A). Induced SR activity was also recorded in the presence of either 10^{-3} M L-glutamate or 10^{-3} M L-aspartate. These agonist concentrations are sufficient to block/desensitize the 'fast' and 'slow' transmitter receptors, respectively[4]. During induced SR activity, neural stimulation would not elicit an excitatory postsynaptic potential (EPSP) (figure 2, C), although recordings from other quiescent fibres in the same 'motor unit'[3] revealed that the nerve was still functioning. Neural stimulation would elicit EPSPs at the start (figure 2, B) and end (figure 2, D and E) of SR activity. Immediately after SR activity, only 'slow' EPSPs could be recorded.

Discussion. SR activity (whether spontaneous or induced) is not caused by nerve stimulation, but bears a marked similarity to cardiac muscle potentials[9] and spontaneous potentials recorded from denervated locust muscle[10]. The insensitivity of SR activity to tetrodotoxin indicates a postsynaptic action which is possibly calcium-dependent. Proctolin and octopamine also have a presynaptic effect at higher concentrations, as indicated by the spontaneous bursting they produce in the motor axons. The postsynaptic action of octopamine and proctolin in producing SR activity is probably mediated by a separate receptor for each substance, as muscles sensitive to one were insensitive to the other. These receptors are probably different from the neurotransmitter receptors, as SR activity could still be recorded when these receptors were blocked.

The low concentrations at which both proctolin and octopamine act, suggest that they might be responsible for spontaneous SR activity. These muscles only receive innervation from 2 motor axons[3], although both axons contain dense-cored vesicles[11,12] which might contain proctolin or octopamine.

The possible function of SR activity is difficult to envisage. During the 3rd instar the innervation changes from a predominantly 'fast' innervation to a predominantly 'slow' innervation[13]. It is interesting, therefore, that following SR activity the 'fast' EPSP is temporarily abolished, leaving only a 'slow' EPSP.

* This work was supported by NIEHS grant No. ES00814 and EPA grant No. R804345.

1 Present address: ICI Plant Protection, Jealotts Hill Research Station, Brackwell, Berks. RG 12 6EY, England.
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Total replacement of blood by an emulsion of fluorocarbon in the rat – water extravasation as a cause of failure

G. Fournier, V. Barrois, C. Girard, D. Robert and L. Foulletier

Pavillon Marcel Berard, Hôpital J. Courmont, F-69310 Pierre-Benite, Laboratoire de Biophysique, UER Lyon Sud Ouest, Chemin du Petit Revoyet, F-69600 Oullins, and Pechiney Ugine Kuhlmann, Rue Henri Moissan, F-69310 Pierre-Benite (France), 2 July 1979

Summary. After exchange transfusion by an emulsion of fluorocarbons (Fluosol 43) in rats, an increase in fluorocarbons and ^{131}I-labelled albumin was observed. These changes suggest a transfer of water from vascular to interstitial space possibly owing to the inability of the emulsion to reproduce the oncotic pressure of normal blood.

Hypovolemia, plasmatic rise in osmolality and viscosity and organ swelling account for the deaths, which are not related to any fluorocarbon-specific toxicity.

Fluorocarbons (FC) can transport important amounts of oxygen and carbon dioxide in solution. Thus FC emulsions have been used as blood substitutes in animals, but few long-term survivals have been achieved[1] in spite of a measured gas transport large enough to sustain life. In a previous work we observed in the rat, after FC total exchange transfusion, an increase in the circulating fluid viscosity and a rise in blood glucose, suggesting hemoconcentration by water extravasation[2].

The aim of this paper is to assess the magnitude of water extravasation and discuss its contribution to the death of animals.

Material and methods. Following a previously reported method[2], 7 rats, weighing from 280 to 350 g, were exchange-transfused with 60 ml of fluosol 43 (Green Cross Corporation) of the following composition: Perfluorotributylamine (FC 43) (20 w/v%); pluronic F68 (2.5 w/v%); NaCl (0.6 w/v%); KCl (0.0034 w/v%); $MgCl_2$ (0.043 w/v%); $CaCl_2$ (0.036 w/v%); $NaHCo_3$(0.21 w/v%); glucose (0.180 w/v%); hydroxyethyl starch (3.0 w/v%); osmolality (280 ± 5 mosm); oncotic pressure (395 mm H_2O); viscosity (3 CP at 37 °C).

In the 7 cases, the exchange-transfusion was achieved in 20 min by the means of indwelling venous and arterial femoral catheters and the hematocrit dropped to less than 3%. The rats were maintained in a 100% oxygen atmosphere. The arterial pressure was monitored by a Statham pressure transducer coupled with a Philips XV1500 monitor. 0.5 ml of ^{131}I-labelled albumin solution (5 µCi/ml) was injected i.v. at time T_0 in 3 treated rats and 2 controls. In the treated group, T_0 corresponded to the end of the exchange transfusion.

1 ml samples of the circulating fluid were taken through the arterial catheter at T_0, 20, 40, 60, 120 and 180 min time. Each sample was replaced by an equal volume of fluosol 43 in the treated animals and by saline in the controls. In 2