of part of the axonal membrane responsible for the G_K "turning on", thus shifting the G_K voltage function along the voltage axis. When protein is removed the remaining structural lipids themselves may generate a high potassium resistance membrane, similar to that observed in artificial protein-lipid membranes¹⁵.

This work was supported by the University of Chile and by the US National Institutes of Health.

EDUARDO ROJAS
ILLANI ATWATER

Laboratory of Biophysics,
Faculty of Sciences,
University of Chile.

Received May 8; revised June 1, 1967.

- ¹ Hodgkin, A. L., and Huxley, A. F., *J. Physiol.*, **117**, 500 (1952).
- ² Tobias, J. M., *Nature*, **203**, 13 (1964).
- ³ Tobias, J. M., *J. Gen. Physiol.*, **43**, 57 (1960).
- ⁴ Rojas, E., and Luxoro, M., *Nature*, **199**, 78 (1963).
- ⁵ Rojas, E., *Proc. US Nat. Acad. Sci.*, **53**, 306 (1965).
- ⁶ Tasaki, I., Watanabe, A., and Singer, I., *Proc. US Nat. Acad. Sci.*, **56**, 1116 (1966).
- ⁷ Takenaka, T., and Yamagishi, S., *Proc. Japan Acad. Sci.*, **42**, 521 (1966).
- ⁸ Nomoto, M., and Narahashi, Y., *J. Biochem.*, Tokyo, **46**, 653 (1959).
- ⁹ Rojas, E., and Ehrenstein, G., *J. Gen. Cell. Comp. Physiol.*, Suppl. 2, **66**, 71 (1965).
- ¹⁰ Rojas, E., and Atwater, I., *Proc. US Nat. Acad. Sci.*, **57**, 1350 (1967).
- ¹¹ Cole, K. S., and Moore, J. W., *J. Gen. Physiol.*, **44**, 123 (1960).
- ¹² Hill, R. L., *Adv. Prot. Chem.*, **20**, 37 (1965).
- ¹³ Hume-Cox, F., Fernandez, H. L., and Smith, B. H., *Biophys. J.*, **6**, 675 (1966).
- ¹⁴ Armstrong, C. M., *J. Gen. Physiol.*, **50**, 491 (1966).
- ¹⁵ Del Castillo, J. A., *Cong. Latino-americano de Ciencias Fisiologicas* (1967).

Comparison between Tetrodotoxin Effects on Excitation in Mammalian Cardiac Fibres and Squid Giant Axons

BECAUSE tetrodotoxin (TTX) was thought to "selectively" block excitatory inward sodium currents in squid giant axons^{1,2}, Dudel *et al.* used TTX as an "independent check" of sodium currents in mammalian Purkinje fibres³. Unfortunately, TTX analysis of membrane currents is no longer possible because recent evidence suggests that neither TTX nor the excitation process is specific for sodium.

Excitation of squid giant axons in media free from sodium is not significantly different from excitation in media containing sodium⁴⁻⁶. "Heart-like" action potentials with typical spike and plateau phases are observed under many conditions⁴⁻⁷, but are characteristic of excitation in external media free from sodium which contain only salts of divalent cations^{5,8}. Such action potentials are usually as easily abolished by TTX as those in media containing sodium^{5,8,9}. The presence of sodium is therefore not essential for excitation, and TTX is not specific for any particular ion. It follows that TTX cannot be used to separate the "sodium current" from other ionic currents during excitation.

Because apparently normal excitation takes place in media free from sodium, and because TTX abolishes the inward current regardless of which ions may be carrying that current, a much more general mechanism than a "sodium-specific carrier" or "sodium-specific pore or channel" is required to understand the excitation process. The "macromolecular model" of the excitation process permits such non-specificity of the participating ions, and can thus account for the effects of TTX in both sodium-containing and sodium-free media^{9,10}. (Extensive descriptions of the macromolecular model are provided elsewhere⁹⁻¹¹.)

IRWIN SINGER

Department of Medicine,
Bronx Municipal Hospital Center,
Bronx, New York.

Received March 28; revised May 15, 1967.

- ¹ Narahashi, T., Moore, J. W., and Scott, D. R., *J. Gen. Physiol.*, **47**, 965 (1964).
- ² Nakamura, Y., Nakajima, S., and Grundfest, H., *J. Gen. Physiol.*, **48**, 985 (1965).
- ³ Dudel, J., Peper, K., Rudel, R., and Trautwein, W., *Nature*, **213**, 296 (1967).
- ⁴ Tasaki, I., Singer, I., and Watanabe, A., *Proc. US Nat. Acad. Sci.*, **54**, 763 (1965).
- ⁵ Tasaki, I., Watanabe, A., and Singer, I., *Proc. US Nat. Acad. Sci.*, **56**, 1116 (1966).
- ⁶ Tasaki, I., Singer, I., and Watanabe, A., *Amer. J. Physiol.*, **211**, 746 (1966).
- ⁷ Adelman, W. J., Dyro, F. M., and Senft, J., *J. Gen. Physiol.*, **48**, 1 (1965).
- ⁸ Watanabe, A., Tasaki, I., Singer, I., and Lerman, L., *Science*, **155**, 95 (1967).
- ⁹ Tasaki, I., and Singer, I., *Ann. NY Acad. Sci.*, **137**, 792 (1966).
- ¹⁰ Singer, I., and Tasaki, I., in *Biological Membranes* (edit. by Chapman, D.) (Academic Press, Inc., London, 1967, in the press).
- ¹¹ Tasaki, I., *Nerve Excitation: a Macromolecular Approach* (Charles C. Thomas, Inc., Fort Lauderdale, Florida, 1967, in the press).

Increase of Cerebral Catecholamines caused by 3,4-Dihydroxyphenylalanine after Inhibition of Peripheral Decarboxylase

N-(DL-seryl)-*N'*-(2,3,4-trihydroxybenzyl)-hydrazine (HCl) (Ro 4-4602) is a strong inhibitor of decarboxylase of aromatic amino-acids *in vivo* and *in vitro*^{1,2}. The drug counteracts the increase of 5-hydroxytryptamine which is induced by 5-hydroxytryptophan less markedly in the brain than in extracerebral organs. Small doses of Ro 4-4602 have even seemed to enhance this transformation in the brain; this effect could not be explained^{3,4}.

This communication shows that small doses of Ro 4-4602 markedly enhance the increase of cerebral catecholamines induced by 3,4-dihydroxyphenylalanine (DOPA) because of a selective inhibition of extracerebral decarboxylase.

Male Wistar rats weighing 250–280 g, fasted for 16 h, were injected intraperitoneally with 50 mg/kg of Ro 4-4602 and 30 min later with 375 γ /rat of DL-2,3-³H-DOPA (specific activity 101.2 mc./mmole). Rats given tritiated DOPA alone served as controls. After decapitation at various times following administration of labelled DOPA, blood plasma was deproteinized with perchloric acid, and the brains and hearts were homogenized in the same acid. The supernatants were brought to pH 8.4 and passed through columns of alumina (Woelm, neutral)⁵. Tritiated DOPA and tritiated catecholamines, eluted with 0.2 normal hydrochloric acid and adsorbed on 'Dowex-50 WX-4' in the sodium ion form (200–400 mesh)⁶, were separated by differential elution using 0.1 normal potassium acetate buffer, pH 6, and 2 normal hydrochloric acid, respectively⁷. In other experiments the action of Ro 4-4602 on the activity of decarboxylase in brain and heart of rats was measured. The procedure involved homogenization of tissue in four volumes of 0.05 molar phosphate buffer, pH 7.4, and incubation of the homogenate in Warburg flasks with 1.5×10^{-5} molar DL-DOPA-1-¹⁴C (specific activity 4.4 mc./mmole) at 37° C for 1 h. The reaction was stopped with 6 normal sulphuric acid, and the labelled carbon dioxide, trapped in the ethanol amine of the centre well, was measured in a liquid scintillation counter after addition of ethyleneglycolmonomethylether. Ro 4-4602 was either injected intraperitoneally 1 h before killing or added to the homogenate. Fig. 1 shows that the ratio of the concentrations of tritiated DOPA in animals treated with Ro 4-4602 versus control animals is increased in brain, blood and heart for at least 4 h. The maximal ratios are 23, 10 and 7.8, respectively. The ratio of the concentration of cerebral tritiated catecholamines in treated animals versus controls shows a continuous increase up to 58 after 4 h. In the heart, however, the tritiated catecholamine ratio remains considerably less than 1. The measurements of decarboxylase activity made using small amounts of labelled substrate confirm and complete the earlier findings with the manometric method². Accordingly, the decarboxylase activity in the brain of animals injected with Ro 4-4602 is not significantly inhibited ($P > 0.05$) up to 100 mg/kg of the drug, whereas in the heart there is 83 per cent inhibition with 50 mg/kg (Fig. 2).