Biochemical Pharmacology, Vol. 24, pp. 1246–1247. Pergamon Press, 1975. Printed in Great Britain

Inhibition of short-circuit current and Na^+, K^+-ATPase in toad bladder by primaquine

(*Received* 8 *October* 1974; *accepted* 13 *December* 1974)

Present understanding of the mode of action of the widely used antimalarial primaquine is limited [1], largely because of the difficulty of working with intracellular *Plasmodia*. Notwithstanding the differences in cellular physiology and biochemistry that no doubt exist between these intracellular parasites and other types of cells, there exists the possibility that the response of other cell types to primaquine may offer clues to its schizonticidal properties as well as offer new insights for the understanding of its side-effects. In this communication, primaquine is shown to inhibit the short-circuit current (SCC) of the toad bladder, which has been shown to be a measure of its net transepithelial sodium flux [2], and to specifically inhibit the sodium-potassium sensitive, magnesium-dependent adenosine triphosphatase (Na^+, K^+-ATPase) that is commonly associated with the transport of sodium [3].

SCC was measured using the method of Ussing and Zerahn [4] on matched sac preparations of toad bladder [5]. The activities of the Na^+, K^+-ATPase and magnesium-dependent adenosine triphosphatase (Mg^{2+}-ATPase) in a homogenate preparation of the toad bladder mucosal epithelial cells were measured using the method of Cortas and Walser [6].

When applied to the bladder in high doses, such as 1–2 mM, primaquine caused a virtual cessation of SCC within 15–20 min. At lower doses, such as 75 μM, the decline in SCC was more gradual, and its initial time course had an exponential form (Fig. 1). After approximately $2\frac{1}{2}$ hr an apparent steady-state of SCC was reached. Partial reversibility of effect was demonstrated by washing the bladders with primaquine-free Ringer's solution (Fig. 1). In early experiments, doses as low as 20 μM were applied to a limited number of bladders. Although some suppression of SCC resulted in each case, the number of experiments done was inadequate to statistically evaluate the significance of the observed changes.

These findings suggested that primaquine might possibly be inhibiting the sodium pump, either directly or by an indirect route. In order to explore this possibility further its effects on the toad bladder Na^+, K^+-ATPase were studied. Primaquine was found to be a potent inhibitor of this enzyme at concentrations ranging from 10^{-10} M to 10^{-4} M (Fig. 2), but surprisingly had no effect on the Mg^{2+}-ATPase activity. Studies with concentrations of primaquine higher than 3×10^{-5} M could not be done because the phosphate anion of the primaquine salt used interfered with the colorimetric assay for phosphate liberated from ATP by the phosphatases [6]. For this reason, it is unknown at present whether full inhibition of the Na^+, K^+-ATPase activity can be achieved with higher doses of primaquine.

From ultrastructural studies of the various stages of the parasite *Plasmodium fallax* grown in embryonic turkey brain cells in culture, it has been found that primaquine will cause swelling of the mitochondria of the parasites as well as dilatation and vesiculation of their endoplasmic reticulum (ER) [7]. Another study has shown that radioactively labeled primaquine is concentrated by the swollen mitochondria over a period of time [8]. Although these data indicate that the mitochondria may be the ultimate target for primaquine, it is possible that primaquine may have also had a deleterious effect on cellular membrane transport. The subsequent inability of the parasites to maintain internal ionic homeostasis may have resulted in the dilated ER and contributed to the swelling of the mitochondria. Such a sequence of morphologic changes in response to disrupted transport of cations at the cellular membrane is known to occur in pathologic studies of various cell types [5, 9].

Several mechanisms have been proposed in the hemolysis of primaquine-sensitive erythrocytes [10, 11]. The present findings raise the possibility of another mechanism for primaquine's hemolytic action. Erythrocytes exposed to primaquine have been shown to lose K^+ and gain Na^+ in an almost 1:1 exchange without undergoing concomitant changes in their redox systems [12]. These findings are compatible with an inhibitory effect of primaquine on the erythrocytic Na^+, K^+-ATPase. It is interesting to

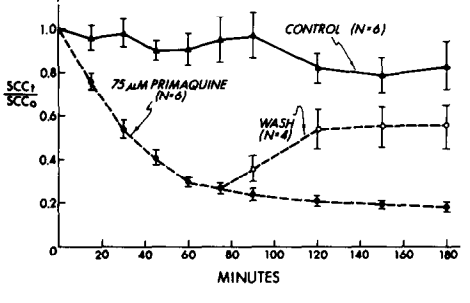

Fig. 1. Time course of inhibition of SCC by 75 μM primaquine phosphate. The ordinate scale is given as the fraction of initial SCC measured at the indicated times. The vertical bars at each point represent $\pm$ 1 S. E. M. In a separate series of experiments, treated bladders were washed with primaquine-free medium after 75 min of exposure. Since the initial time course of decline in SCC was almost identical for the two primaquine-treated groups and since washing was without effect on the controls, the data are combined into a single graph.

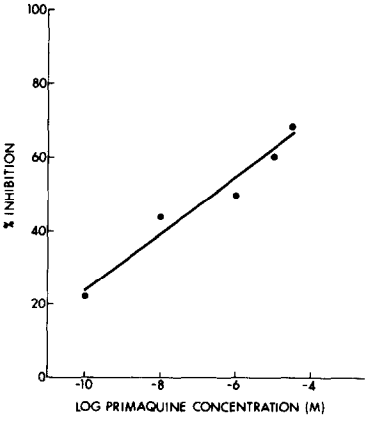

Fig. 2. Per cent inhibition of toad bladder Na^+, K^+-ATPase as a function of the logarithm of primaquine phosphate concentration. Each of the points was run in triplicate. The straight line was computed by means of least squares analysis (r = 0·98).

note that the time course of loss of K^+ in primaquine-exposed erythrocytes [12] remarkably parallels the time course of decline in SCC observed in the present study, an analogy which might be an indication of a similarity in primaquine's effect in the two systems. With the current availability of adequate methods for the study of erythrocytic Na^+, K^+-ATPase, this hypothesis should be possible to test. It is interesting to note that the concentrations required for inhibition of the Na^+, K^+-ATPase in the present study closely approximate the 20 μM serum concentration reached in an individual who daily ingests a hemolytic dose of 30 mg/day [13].

Although the effects of primaquine on Na^+, K^+-ATPase have not previously been tested, quinidine, which has the basic quinoline ring structure in common with primaquine, has been found to inhibit the Na^+, K^+-ATPase activity in preparations of rat skeletal muscle [14] and toad cardiac muscle [15]. It may be that the ability to inhibit the Na^+, K^+-ATPase is a property shared by many of the quinoline ring compounds.

Our results suggest that primaquine directly inhibits active sodium transport in the toad bladder. This effect might be a common action of primaquine in many types of cells and could have a significant role in the hemolysis of primaquine-sensitive mammalian erythrocytes.

Acknowledgements—This work was supported by U. S. Public Health Service Grant 5T01GM00726 and National Institutes of Health Grant GM01678.

Department of Pathology,
Duke University Medical Center,
Durham, N.C. 27710, *U.S.A.*

PAUL J. SIDES
BENJAMIN WITTELS

REFERENCES

1. P. E. Thompson and L. M. Werbel, *Antimalarial Agents: Chemistry and Pharmacology*, p. 100. Academic Press, New York (1972).
2. A. Leaf, J. Anderson and L. B. Page, *J. gen. Physiol.* **41,** 657 (1958).
3. J. C. Skou, *Physiol. Rev.* **45,** 596 (1965).
4. H. H. Ussing and K. Zerahn, *Acta physiol. scand.* **23,** 110 (1951).
5. B. P. Crocker, A. J. Saladino and B. F. Trump, *Am. J. Path.* **59,** 247 (1970).
6. N. Cortas and M. Walser, *Biochim. biophys. Acta* **249,** 181 (1971).
7. M. Aikawa and R. L. Beaudoin, *Milit. Med.* **134,** 986 (1969).
8. M. Aikawa and R. L. Beaudoin, *Expl Parasit.* **27,** 454 (1970).
9. F. L. Ginn, J. D. Shelburne and B. F. Trump, *Am. J. Path.* **53,** 1041 (1968).
10. J. R. DiPalma, in *Drill's Pharmacology in Medicine* (Ed. J. R. DiPalma), 4th Edn, p. 1770. McGraw-Hill, New York (1971).
11. B. Wittels, *Biochim. biophys. Acta* **210,** 74 (1970).
12. R. Weed, J. Eber and A. Rothstein, *J. clin. Invest.* **40,** 130 (1961).
13. D. Berry and P. Hochstein, *Biochem. Med.* **4,** 317 (1970).
14. H. A. Ells, *Proc. Soc. exp. Biol. Med.* **115,** 324 (1964).
15. K. Kennedy and W. Nayler, *Biochim. biophys. Acta* **110,** 174 (1965).