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ACTION OF PRAZIQUANTEL ON CALCIUM TRANSPORT IN HYMENOLEPIS DIMINUTA

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Abstract. Effect of praziquantel on inward and outward Ca^{++} fluxes was investigated in *Hymenolepis diminuta* in glucose supplemented balanced electrolyte solution and under conditions of glucose/Mg^{++} deficiency. The $^{45}Ca^{++}$ uptake in freshly isolated worms presented, generally, a biphasic kinetics. This comprised of an initial fast uptake phase, followed by a continued slower influx. The initial fast kinetics showed insensitivity to or slight stimulation by praziquantel depending on its concentration, and such stimulatory action was particularly prominent under Mg^{++} deficient condition ($P < 0.01$). The subsequent slower $^{45}Ca^{++}$ uptake was, however, markedly inhibited by the drug under both these conditions ($P < 0.01$). Glucose starvation of the worms resulted in abolition of the fast $^{45}Ca^{++}$ influx phase and uniform inhibition by the praziquantel without any indication of initial stimulatory effect ($P < 0.01$). The extrusion of $^{45}Ca^{++}$ from the label preloaded worms was stimulated by praziquantel under all the conditions investigated ($P < 0.01$).

Praziquantel (2-cyclohexylcarbonyl)-1,2,3,6,7,11 b-hexa-hydro-2H-pyrazino(2-1-a) isoquinoline-4-one) is a new antihelminthic agent possesing both antischistosomal (Gönnert and Andrews 1977) and anticestocidal (Thomas and Andrews 1977) activity. The drug induces rapid contractures in representive species of both these groups and this effect is associated with accumulation of external Ca^{++} in *Schistosoma mansoni* (Pax et al. 1978). Fetterer et al. (1980) observed that praziquantel (Pz) causes elevation also of its Mg^{++} content in a high Mg^{++} environment, which transforms the drug induced sustained contracture into transient one; a modification similar to that induced by glucose deficiency in case of high K^{+} induced contractures of isolated guinea pig *Taenia coli* muscles fibres (Urakawa and Holland 1964).

Involvement of environmental Ca^{++} in the mechanism of *S. mansoni* contracture is suggested also from rapid attenuation of this response in a Ca^{++} free medium. Mussie et al. (1982) observed a parallelism between Ca^{++} efflux from these worms in a zero Ca^{++} medium and the responsiveness of their musculature to praziquantel. The mode of action of this drug on the cestode musculature, however, has received scant attention so far. An investigation of Prichard et al. (1982) on *Hymenolepis diminuta* showed inhibition of Ca^{++} influx of praziquantel which is quite opposite to the pattern noted with *S. mansoni*. However, whereas the contracture inducing action manifests itself with great rapidity, the Ca^{++} influx in their experiments was followed after considerable time lag subsequent to drug exposure. The present study more closely examined the change induced in the calcium influx and efflux kinetics by praziquantel in *H. diminuta*, in a glucose supplemented balanced electrolyte medium and in glucose or Mg^{++} deficient environments.

MATERIALS AND METHODS

H. diminuta were collected from the small intestines of albino rats 14 to 16 days p.i. (Voge and Turner 1956). The worms were removed by flushing the intestines with balanced electrolyte solution (BES) (Mettrick and Jackson 1979) followed by thorough washing with the same medium. Only

the anterior portions of the worm strobila (15 to 20 cm) with intact scolices were used in the experiments. Praziquantel was obtained as a kind gift from Dr. H. Thomas of Bayer Institute of Chemotherapy, Wuppertal, West Germany. A stock solution of the drug was prepared in ethanol (1 mg/ml) which was appropriately diluted with BES before addition to the worms.

$^{45}Ca^{++}$ uptake and efflux measurement. The worms were pre-incubated for 45 minutes in a shaking water bath in respective media (details in the legends to the figures) for equilibration. The incubated worms were then divided into several flasks, so that each of them contained two worms of approximately equal size in fresh 5 ml quantity of the same medium supplemented with 10 μCi $^{45}CaCl_2$ (specific activity 50 mCi/g, obtained from BARC, Bombay). These flasks were again mounted in the shaking water bath and a duplicate set was removed at different time intervals. The worms from each sample were washed thrice through quick changes of chilled medium containing 5 mM ethylene glycol-bis(3-aminoethyl ether)N,N'N'-tetra acetic acid (EGTA). The washed worms were blotted dry on filter paper at the indicated time interval and homogenised in 10 % trichloroacetic acid (TCA) in Potter Elvehjem homogeniser and the TCA precipitate was pelleted by centrifugation at 3000×g. The radioactivity was estimated both in TCA precipitate (after its solubilization in 0.2N NaOH) and the supernatant in a Packard Tricarb Liquid Scintillation Counter as reported earlier (Dube and Sagar 1981).

The radioactivity in these estimations being almost exclusively (98 to 100 %) associated with the TCA supernatant (as found by Urakawa and Holland 1964 for *Taenia coli* muscles). The radioactivity found in the supernatant was, therefore, taken as measure of $^{45}Ca^{++}$ incorporation in the worms and was compared in terms of radioactivity per mg protein (latter determined in the corresponding solubilized TCA precipitate by the procedure of Lowry et al. 1951).

For $^{45}Ca^{++}$ efflux measurements, the worms were preloaded with $^{45}Ca^{++}$ by incubation in respective media for 60 minutes. These labelled worms, after being washed similarly, were transferred to a fresh aliquot of the non-radioactive medium of the same composition. The worms were removed from the medium at indicated intervals and the efflux of the radioactivity from these $^{45}Ca^{++}$ preloaded worms was followed both by following the activity in the medium as well as in the TCA supernatants of the worms.

The data presented are generally average of at least two independent repetitions using different batches of the parasites. The results were statistically analysed for S.E. and for chi-square analysis (x^2) to find out the significance difference, if any (Fisher and Yates 1948).

RESULTS

The $^{45}Ca^{++}$ influx in *H. diminuta* presented a biphasic kinetics (Fig. 1). The rate of the radioactivity uptake was thus repeatably and significantly higher during the first few minutes, while a slower progressive increase in the label uptake continued throughout the 60 minutes duration of the experiments ($P < 0.01$). Progressive slow uptake of $^{45}Ca^{++}$ has been reported by Prichard et al. (1982) even 2 hr after the supply of the label, showing that complete specific activity equilibrium between the external and internal $^{45}Ca^{++}$ in these worms is indeed a slow process. The final 60 minute uptake values were significantly inhibited by both the praziquantel concentrations tested. The effect was, however, relatively slight during the first 30 minutes and slight stimulation of the uptake was, in fact, usually indicated at the lower drug level during the early phase.

The Mg^{++} deficiency in the medium caused a decrease in the slope of the initial fast influx, with increase in its duration (Fig. 2). The overall $^{45}Ca^{++}$ uptake under this situation beyond 30 minutes period was, thus considerably higher as compared to that in normal 500 μg/ml glucose supplemented BES (GBES) ($P > 0.01$). Further, the stimulatory action of praziquantel in respect of the initial uptake was particularly prominent under the Mg^{++} deficient condition ($P < 0.01$).

Effect of glucose starvation on ^{45} Ca^{++} influx

Pre-incubation of *H. diminuta* for 4 hr in balanced electrolyte solution in the absence of glucose resulted in abolition of the fast $^{45}Ca^{++}$ influx component of the uptake (Figs. 1 and 3). The praziquantel in these worms caused a more pronounced and uniform inhibition of the radioactivity uptake in the worms ($P < 0.01$).

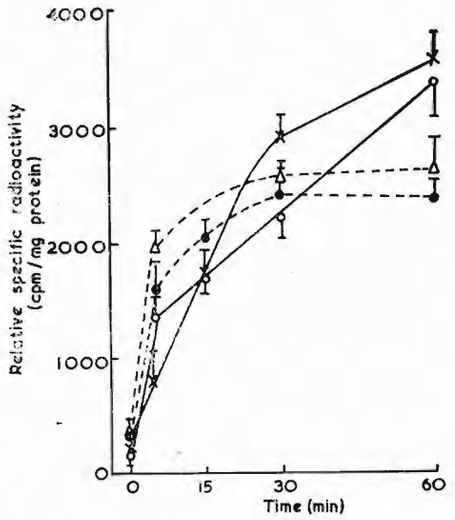

Fig. 1. The effect of praziquantel on $^{45}Ca^{++}$ uptake by *H. diminuta*. Equilibrated worms were incubated in GBES supplemented with $^{45}Ca^{++}$ in absence (o) or presence of 3.13×10^{-6} M (△) or 3.13×10^{-7} M (●) praziquantel.

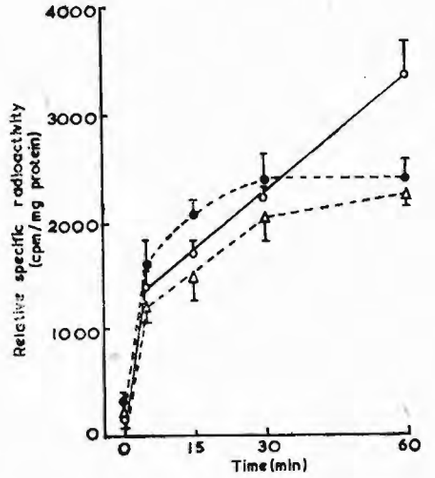

Fig. 2. The effect of praziquantel and Mg^{++} deficiency on $^{45}Ca^{++}$ uptake in *H. diminuta*. The isolated worms after equilibration in normal GBES or Mg^{++} deficient GBES were removed and resuspended in respective $^{45}Ca^{++}$ supplemented media in absence or presence of 3.13×10^{-6} M praziquantel. GBES (o); Mg^{++} deficient GBES (×); GBES + Pz (●); Mg^{++} deficient GBES + Pz (△).

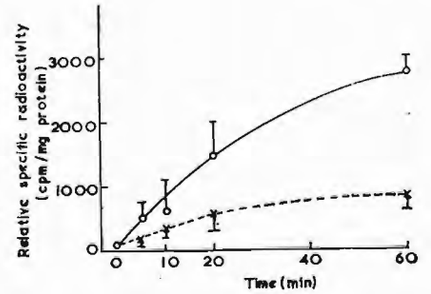

Fig. 3. The effect of praziquantel on $^{45}Ca^{++}$ uptake in glucose starved *H. diminuta*. The worms were pre-incubated for 4 hr in BES and then further incubated in fresh $^{45}Ca^{++}$ supplemented BES in absence (o) or presence (×) of 3.13×10^{-6} M praziquantel.

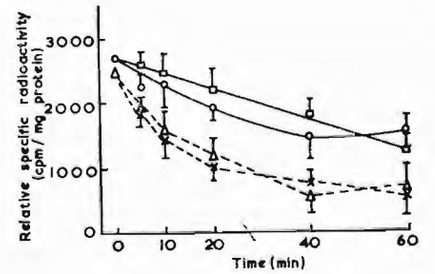

Fig. 4. Effect of Mg^{++} deficiency and praziquantel on the efflux of $^{45}Ca^{++}$ in *H. diminuta*. The isolated worms were labelled in $^{45}Ca^{++}$ supplemented GBES or Mg^{++} deficient GBES for 60 minutes, followed by washing through 4 changes of respective non-radioactive media. The $^{45}Ca^{++}$ preloaded worms were then transferred in respective non-radioacitve media (2 ml) with or without praziquantel to follow $^{45}Ca^{++}$ efflux. GBES (o); Mg^{++} deficient GBES (□); GBES + Pz (△); Mg^{++} deficient GBES + Pz (×). Pz = 3.13×10^{-6} M.

$^{45}Ca^{++}$ efflux from preloaded worms

Calcium efflux estimations both in terms of $^{45}Ca^{++}$ levels in the external media as well as residual radioactivity in the worms indicated an exponential efflux pattern (only the latter data are presented in Figs. 4—5). The pattern of these worms remained more or less altered by glucose deprivation in the medium, although the initial load of radioactivity under this condition was reduced, as expected. Mg^{++} deficiency also did not have any major effect on the efflux pattern, but praziquantel generally stimulated the efflux rates in all the various media examined, viz. the normal GBES, Mg^{++} deficient GBES and the glucose deficient BES ($P < 0.01$).

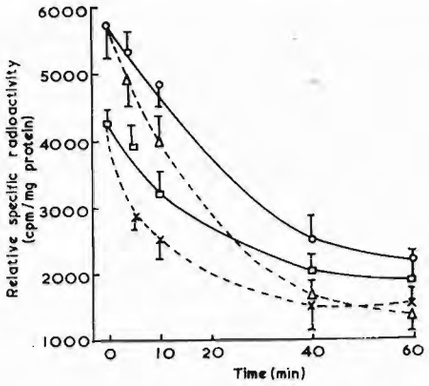

Fig. 5. Effect of glucose starvation and praziquantel (3.13×10^{-6} M) on $^{45}Ca^{++}$ efflux in *H. diminuta*.The isolated worms were pre-incubated in GBES or BES for 4 hr and then again in respective fresh $^{45}Ca^{++}$ supplementd media for 60 min. The labelled worms after washing as usual were transferred to corresponding non-radioactive media with or without praziquantel to follow $^{45}Ca^{++}$ efflux. GBES (○); GBES + Pz (△); BES + Pz (×); BES (□).

DISCUSSION

Prichard et al. (1982) in view of opposite action of praziquantel on $^{45}Ca^{++}$ influx in *H. diminuta* and *S. mansoni* postulated different sources of the threshold mycoplastic Ca^{++} concentrations in the two cases. The contracture inducing Ca^{++} levels according to this hypothesis are derived presumably throught the release of bound internal calcium in the tapeworm, while through uptake of extra-cellular Ca^{++} in the blood flukes. Considerable evidence on the other hand suggests that the tonic contracture, as are characteristically induced by praziquantel (Gönnert and Andrews 1977; Thomas and Andrews 1977), generally involve influx of external calcium not only in *S. mansoni* (Pax et al. 1978) but also in case of several vertebrate muscles (Urakawa and Holland 1964).

Whereas the present experiments with *H. diminuta* confirmed quantitatively opposite influence of praziquantel on calcium influx and efflux, the inhibition of the $^{45}Ca^{--}$ uptake occured generally as a delayed manifestation of its action. The initial fast component of the uptake kinetics, in fact, showed slight stimulation at the lower levels and such response was particularly prominent under the Mg^{++} deficient conditions. The intact *H. diminuta* as used in our experiments, and also those of Prichard et al. (1982) is a complex multicellular biological system which is likely to comprise of a variety of intra-cellular and extra-cellular calcium spaces. The biphasic uptake pattern probably reflects presence of at least two categories of such spaces which differ significantly in their respective equilibration rates for the external $^{45}Ca^{++}$. A biphasic pattern has been observed also for Ca^{++} efflux kinetics in both *H. diminuta* (Prichard et al. 1982) and *S. mansoni* (Mussie et al. 1982), which again suggests presence of different calcium compartments in these worms. The flow of external calcium into such distinct compartments may conceivably have differential praziquantel sensitivity. This might account for the delayed manifestation of the inhibitory response towards $^{45}Ca^{++}$ uptake, despite the known rapidity of its contracture inducing action, which might suggest its ready permeability into the worm. Such a complex pattern of action, may mask, to a greater or lesser extent, the rapid spurt in permeability of external Ca^{++} in the parasite muscles due to praziquantel as evident in *S. mansoni* (Pax et al. 1978). This possibility must be considered before postulating qualitatively different sources of Ca^{++} in the contracture inducing action of the drug in these two species (Prichard et al. 1982).

The rapid uptake component of $^{45}Ca^{++}$ influx was attenuated when the worms were subjected to glucose starvation. This suggests its dependence on the energy reserves in the parasites, an interference supported by the presence of high Ca^{++} ATPase activity in tegumental brush border of *H. diminuta* (Prichard et al. 1982). This mechanism may have a role in concentrating environmental calcium into some *H. diminuta* compartments even when the external Ca^{++} levels are very low and might account for relative insensitivity of the contracture inducing action of praziquantel to medium, Ca^{++} concentration as reported by Prichard et al. (1982). Alternately these worms may have additional Ca^{++} reserves whose content can find its access into appropriate spaces, whereby the contracture inducing drug action can operate even under the Ca^{++} deficient condition in the medium. An investigation of the effect of glucose starvation on the contracture inducing response of praziquantel and further analysis of its effect on Ca^{++} transport in isolated tissue of the parasite will be necessary to resolve this question.

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ДЕЙСТВИЕ ПРАЗИКВАНТЕЛА НА ТРАНСПОРТ КАЛЬЦИЯ У *HYMENOLEPIS DIMINUTA*

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Резюме. Изучали действие празиквантела на проникание Ca^{++} вовнутрь и наружу у *Hymenolepis diminuta* в растворе электролитов с глюкозой и при недостатке глюкозы/Mg^{++}. Поглощение $^{45}Ca^{++}$ у червей непосредственно после их выделения представляло собой в общем двухфазную кинетику: первичную фазу быстрого проникания вовнутрь и последующую фазу непрерывного, более медленного вытекания наружу. Празиквантел или не оказывал влияния или оказывал только небольшое стимулирующее действие на первичную быструю фазу, в зависимости от его концентрации. Стимулирующее влияние было особенно выразительно в условиях недостатка Mg^{++} ($P < 0,01$). Последующая медленная фаза поглощения $^{45}Ca^{++}$ значительно ингибировалась празиквантелом при обоих условиях ($P < 0,01$). При недостатке глюкозы у паразитов быстрая фаза поглощения $^{45}Ca^{++}$ не осуществилась и ингибирование празиквантелом протекало без первоначального стимулирующего действия ($P < 0,01$). Экструзия $^{45}Ca^{++}$ из меченых червей стимулировалась празиквантелом во всех изучаемых условиях ($P < 0,01$).

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