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Praziquantel impairs the ability of exogenous serotonin to stimulate carbohydrate metabolism in intact *Schistosoma mansoni*

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Abstract. Praziquantel (PZQ) (Droncit, Biltricide) at 10 μ*M* completely abolishes the stimulatory effect of serotonin on glucose uptake and lactate excretion of *Schistosoma mansoni*. Fluoxetine (FXT) exerts similar effects on the serotonin-induced stimulation of glucose uptake and lactate excretion, however, at 100-fold higher concentrations. In comparison with PZQ, which is inhibitory at 10 μ*M*, FXT and other amphiphilic cationic drugs (amitriptyline, propranolol, imipramine, chlorpromazine) inhibit glucose uptake or lactate excretion in schistosomes at 1 m*M*; the strongest inhibitor is FXT. Glycogen breakdown is maximally stimulated by PZQ in the absence or presence of serotonin. There is an additive effect of 50 μ*M* chlorpromazine or FXT and 0.01 to 0.1 μ*M* PZQ on glycogen breakdown. The rate of sodium-sensitive or insensitive serotonin uptake in *Schistosoma mansoni* is reduced by 10 μ*M* PZQ by about 40%, as is the sodium-sensitive excretion of serotonin. The results show that PZQ interferes with the ability of serotonin to stimulate carbohydrate metabolism. The possibility that PZQ may act through an effect on tegumental integrity is discussed.

Serotonin has a stimulatory effect on glucose uptake and glycogenolysis (Rahman and Mettrick 1985) in the adult trematode *Schistosoma mansoni*, which is a homolactic fermenter in vitro (Czok et al. 1975; Bueding and Fisher 1982; Bueding 1950). It is likely that this neurotransmitter acts via cAMP in *S. mansoni*, as has been shown for *Fasciola hepatica* (Mansour 1979). In the presence of praziquantel (PZQ) glycogen breakdown is stimulated in *S. mansoni* (Andrews 1985) as well as in *Hymenolepis diminuta* (Andrews and Thomas 1979). Thus, it is tempting to speculate that PZQ might act like serotonin either directly by interfering with serotonin binding sites or indirectly by interfering with external membranes of the parasites thus disturbing the serotonin uptake system.

Therefore, experiments were performed with *S. mansoni* to observe the influence of PZQ, fluoxetine (FXT) and the amphiphilic cationic drugs amitriptyline, propranolol, imipramine and chlorpromazine on the stimulatory properties of serotonin. These drugs are known to be membrane-active (Seeman 1966; Seeman 1972) and, as FXT is a very selective serotonin uptake inhibitor (Fuller and Wong 1977), it should be possible to decide whether the mode of action of PZQ on serotonin binding sites is largely direct or indirect.

Materials and methods

In this study 8-week-old *S. mansoni* pairs were isolated from the portal veins of CF1/W74 mice that had been killed with ether to dislodge the worms from the mesenteric veins. The mice had been infected by subcutaneous injection of 200 cercariae obtained from 8-week-old *Biomphalaria glabrata* snails.

Compounds. Chlorpromazine, amitriptyline, imipramine, propranolol and serotonin were obtained from SIGMA (München). Fluoxetine was a gift from Lilly Research Laboratories (Indianapolis, USA).

Incubation conditions with non-radioactive compounds. After isolation, the parasites were rinsed in medium TC 199 with Earle's balanced salt solution (SERVA, Heidelberg, No. 47000A) containing 10% calf serum and 100 μg/ml mezlocillin (Baypen). Groups of 15 pairs were incubated aerobically in 1.5 ml medium for 120 min. Drugs were dissolved in ethanol and added to the medium. In the combination experiments, the parasites were preincubated in the presence of one drug for 30 min and after this time a second drug was added for a further 120 min incubation period. Controls were run with or without the preincubation. The maximal ethanol concentration in all incubations was

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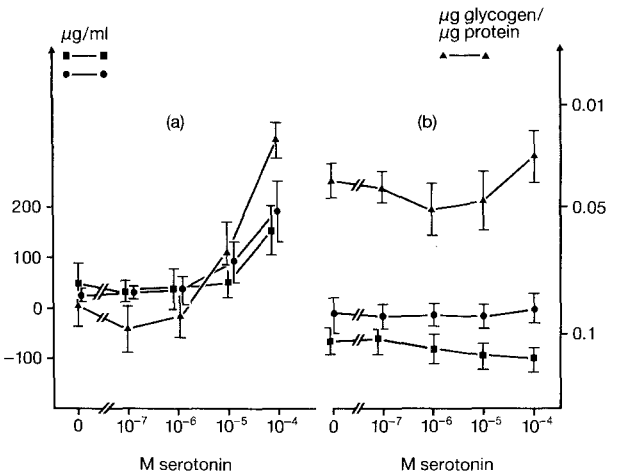

Fig. 1 a, b. Glucose uptake (µg/ml; ■–■), lactate excretion (µg/ml; ●–●) and glycogen breakdown (µg glycogen/µg protein; ▲–▲) of 15 pairs of *Schistosoma mansoni* after 120 min incubation at different serotonin concentrations relative to control. **a** without preincubation of praziquantel and **b** with preincubation for 30 min in presence of 10 µ*M* praziquantel. Each *point* is the mean value of 5 incubations each with 15 pairs of *S. mansoni*

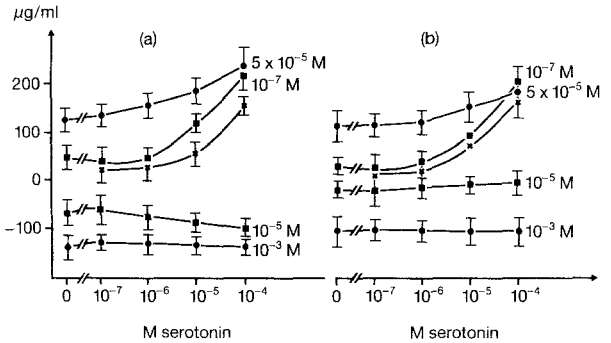

Fig. 2. a Glucose uptake (µg/ml) of 15 pairs of *S. mansoni* after 120 min incubation at different concentrations of serotonin in presence of 50 µ*M* or 1 m*M* fluoxetine (●–●) or 0.1 µ*M* or 10 µ*M* praziquantel (■–■). Serotonin control (x–x). **b** Lactate excretion (µg/ml). Experimental conditions and explanations are the same as for glucose uptake. Each *point* is the mean value of 5 incubations each with 15 pairs of *S. mansoni*

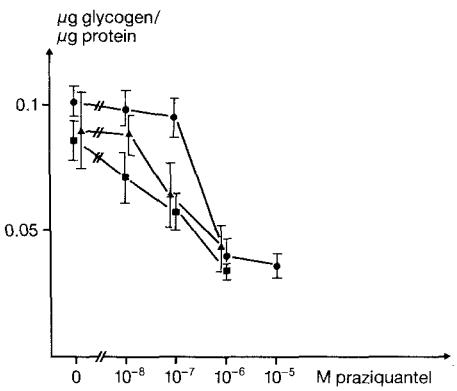

Fig. 3. Glycogen breakdown (µg glycogen/µg protein) of 15 pairs of *S. mansoni* after 120 min incubation at different praziquantel concentrations. Parasites were preincubated without (●–●) or with 50 µ*M* fluoxetine (▲–▲) or 50 µ*M* chlorpromazine (■–■). Each *point* is the mean value of 5 incubations each with 15 pairs of *S. mansoni*

1%. Glucose and lactate estimations were performed using Biochemica test combinations (Boehringer, Mannheim). Glycogen was determined according to van Handel (1965), protein according to Hartree's (1972) modification of the Lowry method.

Incubation with [^3H] serotonin. The [^{3}H] serotonin (specific activity: 500 GBq/mmol) was purchased from Amersham Buchler, Braunschweig, FRG. All incubations were performed at 37° C in a modified Krebs-Ringer-solution (Webb 1985). Sodium-free medium contained an equimolar amount of sucrose replacing NaCl.

Serotonin uptake experiments. In these experiments, 20 pairs of schistosomes were incubated in the modified Krebs-Ringer-solution (1 ml) containing 1 µ*M* [^{3}H] serotonin (specific activity: 500 GBq/mmol) in the presence or absence of sodium or 10 µ*M* PZQ. After 3, 6, 9, 12 or 15 min, the incubation was terminated by gentle centrifugation (30 s, 100 *g*), after which the schistosomes were washed twice and placed in vials containing tissue solubilizer and Filter count (Packard) scintillator.

Serotonin release. Here 20 pairs of schistosomes were incubated in the modified Krebs-Ringer-solution (1 ml) in the presence of sodium and [^{3}H] serotonin (specific activity: 500 GBq/mmol) for 15 min. After centrifugation and two washes the schistosomes were placed in a second vial filled with medium (1 ml; normal or sodium-free) with or without the addition of 10 µ*M* PZQ. After 3, 6, 9, 12 or 15 min an aliquot of 250 µl was taken and measured for radioactivity.

Results

Glucose uptake, lactate excretion and glycogen breakdown were stimulated by 100 µ*M* serotonin (Fig. 1 a). However, when parasites were incubated with 10 µ*M* PZQ for 2 h glucose uptake was inhibited compared to controls, and lactate excretion remained unchanged (Fig. 1 b). Furthermore, both these processes could no longer be stimulated by the addition of serotonin. Moreover, glycogen breakdown was maximally stimulated in the presence of 10 µ*M* PZQ, irrespective of whether serotonin was present or absent (Fig. 1 b).

Glucose uptake (Fig. 2 a) and lactate excretion (Fig. 2 b) were also inhibited by 1 m*M* FXT. Again, as in the case of 10 µ*M* PZQ, serotonin had no further stimulatory effect in the presence of 1 m*M* FXT (Fig. 2). Glycogen breakdown in *S. mansoni* was stimulated maximally above a concentration of 1 µ*M* PZQ (Fig. 3). At 0.1 µ*M* PZQ, no enhanced glycogen breakdown could be observed compared with controls. However, when 50 µ*M* chlorpromazine or FXT were present simultaneously, a stimulation of glycogen breakdown could be detected, even at PZQ concentrations as low as 0.1 µ*M* or 0.01 µ*M* (Fig. 3).

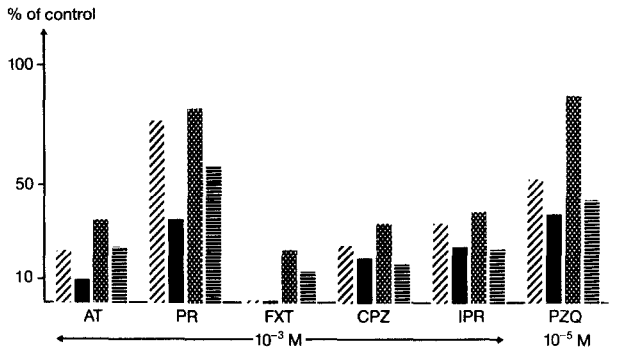

Fig. 4. Glucose uptake (▨, ■) and lactate excretion (▩, ▤) of *S. mansoni* as percentage of control in presence of different drugs: 1 m*M* amitriptyline (AT), propranolol (PR), fluoxetine (FXT), chlorpromazine (CPZ), imipramine (IPR) or 10 μ*M* praziquantel (PZQ), in the presence (■, ▤) or the absence (▨, ▩) of 100 μ*M* serotonin. Each *point* is the mean values of two experiments

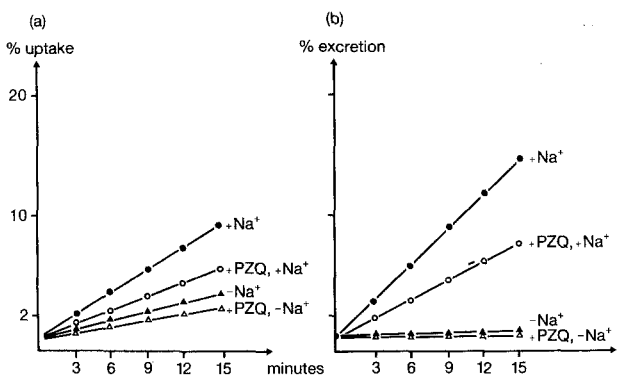

Fig. 5a, b. Uptake (**a**) or excretion (**b**) of (^{3}H)-serotonin of 20 pairs of *S. mansoni* within 15 min in presence (●. ○) or absence (▲, △) of sodium and in the presence of 10 μ*M* praziquantel (*open circles* or *triangles*) or absence of praziquantel (*closed circles* or *triangles*). Each *point* is the mean value of two experiments. For detailed experimental conditions see Materials and methods

The different amphiphilic cationic drugs amitriptyline, propranolol, fluoxetine, chlorpromazine or imipramine inhibited glucose uptake or lactate excretion at 1 m*M*, whereas PZQ exerted its effects at 10 μ*M* (Fig. 4). In the presence of 100 μ*M* serotonin, the inhibition relative to controls was significantly greater than in its absence (Fig. 4). The strongest inhibitor among the five drugs was FXT.

Serotonin uptake by *S. mansoni* was strongly sodium-dependent (Fig. 5a). 10 μ*M* PZQ inhibited the rate of uptake by almost 40% (Fig. 5a) both in the presence and absence of sodium. The excretion of serotonin was largely sodium dependent (Fig. 5b). Again the rate of excretion was reduced by 10 μ*M* PZQ by about 40%. In the absence of sodium, serotonin excretion was very low; no further reduction by PZQ could be measured.

Discussion

In the adult trematode *S. mansoni*, the carbohydrate metabolism can be stimulated by serotonin (Rahman and Mettrick 1985) (Fig. 1 a). The stimulation can be detected in glucose uptake, lactate excretion and glycogen breakdown. The metabolic stimulation is accompainied by an enhanced motility of the parasites. Recently, an operative Krebs cycle has been demonstrated in *S. mansoni* (van Oordt et al. 1985) leading to considerable production of CO_2 from glucose consumption. However, it is not the intention of the experiments described here to investigate the detailed carbohydrate metabolism, but to compare the influence of different drugs on the metabolism. Therefore we chose the three parameters – glucose uptake, glycogen breakdown and lactate excretion – to simplify estimation. In the presence of 10 μ*M* PZQ, the carbohydrate metabolism is disturbed. There is an inhibition of glucose uptake, while lactate excretion remains normal, but both processes can no longer be stimulated by serotonin, glycogen breakdown is maximally induced by PZQ, even in the absence of serotonin (Fig. 1 b) These results show that exogenous serotonin is without effect on the action of PZQ on the carbohydrate metabolism of *S. mansoni*. FXT, a PZQ antagonist in *S. mansoni* with respect to muscle tension and Ca^{++}-influx (Pax et al. 1979), exerts effects similar to PZQ on serotonin-induced glucose uptake and lactate excretion, although at 100-fold higher concentrations than PZQ (Fig. 2). Even the addition of 100 μ*M* serotonin did not increase glucose uptake and lactate excretion at 1 m*M* FXT. At lower drug concentrations (0.1 μ*M* PZQ or 50 μ*M* FXT), however, both metabolic processes could be stimulated by 100 μ*M* serotonin (Fig. 2). The stimulation by serotonin was greater in the presence of 0.1 μ*M* PZQ than 50 μ*M* FXT. The reason for this is presumably that FXT specifically inhibits serotonin uptake, as has recently been shown in schistosomulae of *S. mansoni* (Catto and Ottesen 1979) and in *Hymenolepis diminuta* (Webb 1985) (Fig. 2).

PZQ alone leads to a full stimulation of glycogen breakdown at 1 μ*M* (Fig. 3), whereas at 0.1 μ*M* it is ineffective. Interestingly, other biochemical and physiological events are induced at 1 μ*M* and higher PZQ concentrations viz. a contraction of the parasites (Pax et al. 1978; Andrews et al. 1983), severe tegumental disruption (Andrews et al. 1983; Andrews 1985; Becker et al. 1980) and an inhibition of glucose uptake and lactate excretion (Andrews 1985). Furthermore the

Ca^{++}-permeability is enhanced (Pax et al. 1979). However, the threshold concentration of PZQ, which is between 0.1 and 1 μM, is reduced when FXT or chlorpromazine are also present; very small concentrations of PZQ (0.01 up to 0.1 μM) can stimulate glycogen breakdown in *S. mansoni* in the presence of 50 μM FXT or chlorpromazine, while PZQ alone does not exhibit this effect (Fig. 3). At 50 μM, chlorpromazine and other amphiphilic cationic drugs are known to increase the permeability of membranes to ions and non-electrolytes (Seeman 1966; Seeman 1972). Thus, it is possible that the otherwise ineffective low concentrations of PZQ are sufficient to stimulate glycogen breakdown (Fig. 3) when an amphiphilic cationic drug is simultaneously present.

The different amphiphilic cationic drugs and PZQ exert qualitatively similar effects on glucose uptake or lactate excretion in the absence and in the presence of 100 μM serotonin (Fig. 4). In both instances, however, PZQ is 100-fold more effective than the other five drugs.

At 10 μM, PZQ acts as an inhibitor of serotonin uptake and excretion in the presence and in the absence of sodium (Fig. 5). At the same concentrations, FXT acts as a serotonin uptake inhibitor in *Hymenolepis diminuta* (Webb 1985). However, the mechanism of inhibition by FXT and PZQ may well be different. FXT is a very selective serotonin uptake inhibitor (Fuller and Wong 1977), but stimulates glucose uptake in our experiments. PZQ on the other hand inhibits both processes (Fig. 1 b, Fig. 5).

Recently it has been shown that 10 μM PZQ inhibits several tegumental enzymes such as Ca^{++}-ATPase of *S. mansoni* (Nechay et al. 1980), cAMP-phosphodiesterase, alkaline phosphatase, 5′-nucleotidase and other brush-border enzymes of *Cotugnia digonopora* (Pampori et al. 1985). The authors conclude that these effects were not caused by direct inhibition of the enzymes by PZQ, but rather by perturbance of the tegumental environment. We also believe that glucose and serotonin uptake, which are protein mediated processes requiring intact membranes, are disturbed by PZQ in the same way.

References

Andrews P (1985) Praziquantel: mechanisms of anti-schistosomal activity. Pharmacol Ther 28:129–156

Andrews P, Thomas H (1979) The effect of praziquantel on *Hymenolepis diminuta* in vitro. Trop Med Parasitol 30:391–400

Andrews P, Thomas H, Pohlke R, Seubert J (1983) Praziquantel. Med Res Rev 3:147–200

Becker B, Mehlhorn H, Andrews P, Thomas H, Eckert J (1980) Light and electron microscopic studies on the effect of praziquantel on *Schistosoma mansoni, Dicrocoelium dendriticum* and *Fasciola hepatica* (Trematoda) in vitro. Z Parasitenkd 63:113–128

Bueding E (1950) Carbohydrate metabolism of *Schistosoma mansoni*. J Gen Physiol 33:475–495

Bueding E, Fisher J (1982) Metabolic requirements of schistosomes. J Parasitol 68:208–212

Catto BA, Ottesen EA (1979) Serotonin uptake in schistosomules of *Schistosoma mansoni* Comp Biochem Physiol [C] 63:235–242

Czok R, Czifer S, Jelinic B (1975) Glycogenmetabolismus in *Schistosoma mansoni*. Wien Tierärztl Mschr 62:249–254

Fuller RW, Wong DF (1977) Inhibition of serotonin reuptake. Fed Proc 36:2154–2158

Hartree EF (1972) Determination of protein; a modification of the Lowry method that gives a linear photometric response. Anal Biochem 48:422–427

Mansour TE (1979) Chemotherapy of parasitic worms: new biochemical strategies. Science 205:462–469

Nechay BR, Hillman GR, Dotson MJ (1980) Properties and drug sensitivity of adenosine triphosphatases from *Schistosoma mansoni*. J Parasitol 66:596–600

Pampori NA, Singh G, Srivastava VML (1985) Enzymes of isolates brush border membrane of *Cotugnia digonopora* and their insensitivity to anthelmintics in vitro. Vet Parasitol 18:13–19

Pax RA, Bennett JL, Fetterer R (1978) A benzodiazepine derivative and praziquantel: effects on musculature of *Schistosoma mansoni* and *Schistosoma japonicum*. Naunyn Schmiedebergs Arch Pharmacol 304:309–315

Pax RA, Fetterer R, Bennett JL (1979) Effects of fluoxetine and imipramine on male *Schistosoma mansoni*. Comp Biochem Physiol [C] 64:123–127

Rahman MS, Mettrick DF (1985) *Schistosoma mansoni*, effects of in vitro serotonin (5-HT) on aerobic and anaerobic carbohydrate metabolism. Exp Parasitol 60:10–17

Seeman P (1966) Membrane stabilization by drugs: tranquilizers, steroids and anesthetics. Int Rev Neurobiol 9:145–221

Seeman P (1972) The membrane actions of anesthetics and tranquilizers. Pharmacol Rev 24:583–655

Van Handel LE (1965) Estimation of glycogen in small amounts of tissue. Anal Biochem 22:256–265

Van Oordt BEP, van den Heuvel JM, Tielens AGM, van den Bergh SG (1985) The energy production of the adult *Schistosoma mansoni* is for a large part aerobic. Mol Biochem Parasitol 16:117–126

Webb RA (1985) The uptake and metabolism of 5-hydroxytryptamine by tissue slices of the cestode *Hymenolepsis diminuta*. Comp Biochem Physiol [C] 80:305–312

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