Anesthesia of *Biomphalaria* spp. (Mollusca, Gastropoda): Sodium Pentobarbital is the Drug of Choice

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The anesthetic effect of some water-soluble anesthesic or narcotic drugs currently used in mice was tested in molluscs of the Biomphalaria *genus.*

Sodium thiopental was very toxic to the snails resulting in high rates of mortality in all the treatment schedules tested. Cetamine base, at concentration of 0.25 mg/ml of water, resulted in partial snail anesthesia (40% of snails were anesthetized) only after 20 h of exposition. The association of Cetamine base with Tiazine chloridrate did not improve the anesthesic effect, and higher concentrations of these drugs were toxic to the snails.

Sodium pentobarbital at 0.4 mg/ml in water for 8 h was the best treatment schedule to anesthetize Biomphalaria *snails. In this schedule, the snails were anesthetized without any toxic effect. The procedure provides a powerful tool for in vivo studies that demande a complete state of snail anesthesia.*

Key words: sodium pentobarbital - *Biomphalaria* - anesthesia - mollusc inoculation

The study of systematic, physiology, pharmacology and host-parasite relationship is compound by the lack of an efficient, simple and inexpensive method for anesthesia or muscle relaxing of gastropod molluscs.

Pan (1958) demonstrated that snail exposition to a menthol solution produce muscle relaxation in pulmonate molluscs, allowing a better tissue fixation aiming histological analysis. Michelson (1958) demonstrated that the best snail distension and immobilization was obtained through the immersion of snail in urethan aqueous solution. This methodology has been largely used in many mollusc species, however the technique does not permit snail manipulation, which demands a complete state of anesthesia. Runhan et al. (1965) concluded that anesthetic or narcotic drugs have a variable effect on mollusc, depending on the mollusc species tested, the drug concentration and the exposition time. Planorbidae molluscs are routinely relaxed by sodium pentobarbital in taxonomical and systematical studies at the Department of Malacology of Instituto Oswaldo Cruz (Dr W Lobato Paraense, pers. comm.)

MATERIALS AND METHODS

Water-soluble drugs that had a reported anesthetic and/or narcotic effect on mice were selected to be tested in these experiments. Sodium pentobarbital (Hypnol®, Cristália, Brazil) was tested at 0.1, 0.2, 0.4, 0.8, 1.6 mg/ml; sodium thiopental (Thionebutal®, Abbot, Brazil) at 0.048, 2.5, 3.3 and 5 mg/ml; cetamine base (Ketalar®, Parke-Davis, Brazil) at 0.25, 0.5 and 0.75 mg/ml associated with chloridate 2-(2,6 xilidine)-5,6-dehidro-4HI, 3 tiazine (Rompun®, Bayer, Brazil) in a proportion of six parts of Ketalar to one part of Rompun as recommended for mice.

To each drug solution, ten *Biomphalaria tenagophila*, measuring 12-14 mm of diameter, were placed into a 500 ml beaker with 100 ml dechlorinated water containing the testing drug. The snails were kept into the drug solution and examined after 3, 5, 8 and 20 h. The number of snail in each treatment that showed no effect, relaxation, anesthesia, or death was observed. A snail was considered relaxed if it had the cephalopodal region completely exposed but the animal slowly retracted when touched with a seringle needle. An anesthetized snail was defined as an animal that does not retract to the shell when injected, in cephalopodal region, with 10 µl of PBS through a 21-G needle. After the inoculation the snails were

This work was partially supported by Fapemig and CNPq/Pronex.
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++CNPq fellowship
Received 5 July 2000
Accepted 27 September 2000

transferred to another flask with water and the mortality was checked 24 h after inoculation.

RESULTS AND DISCUSSION

B. tenagophila exposed to all the tested concentrations of sodium thiopental showed immediate retraction, hemolymph lost and death. Snail retraction was also observed with cetamine alone at concentrations of 0.5 and 0.75 mg/ml, or when associated with tiazine chloridrate.

Cetamine at 0.25 mg/ml was not toxic to the snails, however the relaxation occurred only after 8 h of exposition in 60% of the snails and complete anesthesia was observed in 40% of the 20 h exposed snails. All the cetamine anesthetized snails survived to the PBS inoculation. The association of 0.25 mg/ml of cetamine with 0.04 mg/ml of tiazine chloridrate did not improve the anesthetic effect obtained with cetamine alone, however tiazine chloridrate at concentrations higher than 0.1 mg/ml were toxic to *B. tenagophila*.

Less than 10 and 20% of the snails exposed to 0.1 and 0.2 mg/ml of sodium pentobarbital showed muscle relaxation after 8 h (Table-A). However, all the snails exposed to 0.4 mg/ml of sodium pentobarbital were completely anesthetized after 8 h, allowing experimental manipulation such as PBS inoculation. Snail exposition to higher drug concentration such as 0.8 and 1.6 mg/ml leads to snail mortality (Table-A).

The results showed that snail exposition to sodium pentobarbital at 0.4 mg/ml for 8 h was the most effective procedure to anesthetize *B. tenagophila*, with a very low mortality rate (Table-B). The same treatment was also effective to anesthetize *B. glabrata* in our laboratory. The Hipnol® solution can be re-used 4 to 5 times, if filtered and kept at 4ºC. The treatment resulted in complete distention and relaxation of the snail muscle, allowing experimental manipulation *in vivo*, such as transplantation of internal organs, transference of hemolymph or cells from *Schistosoma mansoni* resistant snail strains to susceptible strains and other in vivo interference that may help to understand the interaction of *S. mansoni* and *Biomphalaria* spp.

ACKNOWLEDGEMENTS

To the GIDE (Schistosomiasis Research Unit) staff, for the technical support in the experiments.

REFERENCES

Michelson EH 1958. A method for relaxation and immobilitation of pulmonate snails. *Trans Am Micros Soc 77*: 316-319.

Pan CT 1958. The general histology and topographic microanatomy of *Australorbis glabratus*. *Bull Mus Comp Zool Harvard 119*: 237-299.

Runham NW, Isarankura K, Smith BJ 1965. Methods for narcotizing and anaesthetizing gastropods. *Malacologia 2*: 231-238.

TABLE

Effect of sodium pentobarbital on *Biomphalaria tenagophila*

A: snails analyzed after 8 h of exposition to different drug concentrations

Concentrations (mg/ml)	No effect (%)	Relaxation (%)	Anesthesia (%)	Death (%)
0.1	90	10	0	0
0.2	80	20	0	0
0.4	0	0	100	0
0.8	0	0	80	20
1.6	0	0	30	70

B: snails exposed to sodium pentobarbitol solution (0.4 mg/ml) and analyzed at different exposition time

Exposition time (h)	Without effect (%)	Relaxation (%)	Anesthesia (%)	Death (%)
3	100	0	0	0
5	0	100	0	0
8	0	0	100	0
20	0	0	0	100