Effect of strychnine on the rat electroretinogram

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We have recently reported that intravitreal injection of strychnine (minimum effective dose 0·08 μmol/10 μl each eye) caused a reversible negative deflection (a-like wave) in response to low intensity flash stimuli (about 1·0 log unit above dark threshold) in the rat electroretinogram (ERG) (Pong & Graham, 1976). This effect is specific to glycine-antagonists and is different from the characteristic rhythmic potentials induced by GABA-antagonists (Graham & Pong, 1972; Pong & Graham, 1976). The quaternization of the glycine-antagonists abolished the strychnine-like activity and endowed the ability to induce the rhythmic potentials characteristic of the GABA-antagonists. Although the interpretation of these findings is difficult due to the complexity of the vertebrate ERG, further investigation seemed to be warranted. We have therefore studied the dose-dependent time course effect of strychnine on the amplitude of the b-wave of the rat ERG in response to a constant light stimulus. The results indicate that early claims of the possible beneficial effect of strychnine in amblyopia (cf. Grant, 1974) seem to be not without substance.

All experiments were performed on anaesthetized (sodium pentobarbitone, 50 mg kg^{-1}, i.p.) male albino rats (230–350 g) which were dark-adapted and fasted overnight. The recording of the ERG was essentially as described by Cone (1963) with slight modification (Pong & Graham, 1976). ERGs in response to different intensities of light stimulus were compared before, and at various intervals after, the intravitreal administration of 10 μl saline or drug solution. The injection was made with a 30-gauge needle attached to a Hamilton syringe. The top tracings (time 0 min) of Fig. 1 show the ERGs of the control rats in response to flash stimuli at four intensities before any injection. The amplitude of the b-wave of the ERG in the saline-injected rat (Fig. 1A) gradually decreased within 40 min after injection. That of strychnine-injected rats (Fig. 1B), however, rapidly decreased with a peak effect at about 10 min and then recovered rather rapidly almost to the original height at 40 min. In addition, strychnine induced the appearance of an a-like wave in response to the lower flash stimuli (N.D. No. 4.0 and 3.0) during the peak effect. This is of particular interest, because it is well documented that the a-wave threshold is about 2 to 3 log units *above* the b-wave threshold (Dowling, 1967) and strychnine markedly reduced the a-like wave threshold to even lower than the b-wave threshold (i.e., about 2 to 3 orders of magnitude increase in sensitivity).

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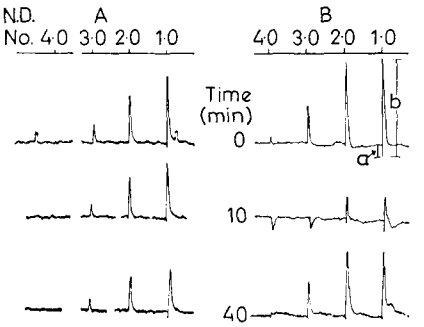

FIG. 1. Time course effects of A: saline and B: strychnine (0·3 μmol/10 μl each eye) on rat ERG in response to different flash stimuli. Time 0 min indicates ERGs before injection. The flash stimuli with filter density of N. D. No. 4·0 were about 0–0·5 log unit above dark threshold. The calibration signal is 100 μV amplitude and 100 ms duration.

These strychnine effects were not seen at a lower dose (e.g., 0·06 μmol/10 μl each eye) which, however, maintained the b-wave at a higher amplitude than that of the saline-injected control. Thus, the effect of strychnine on the b-wave amplitude was apparently dose-dependent.

Since the effect of strychnine on the ERG is most characteristic at a light intensity with a filter of 3·0 (Fig. 1B), the b-wave amplitudes in response to that light intensity after different doses of strychnine were compared at the time of peak effect, i.e. 10 min after injection. The b-wave amplitude was measured arbitrarily

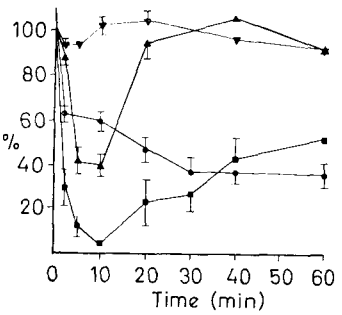

FIG. 2. Dose-response of the effect of strychnine (μmol each eye) on the amplitude of the b-wave (%) in response to constant flash stimuli (filter intensity of 3·0 and stimulus duration of 40 ms) as a function of time (min). The amplitude before injection served as 100%. Each point represents the mean of not less than 5 observations and is given with s.e.m. ● Saline; ▼ 0·06; ▲ 0·10; ■ 0·15 μmol strychnine each eye.

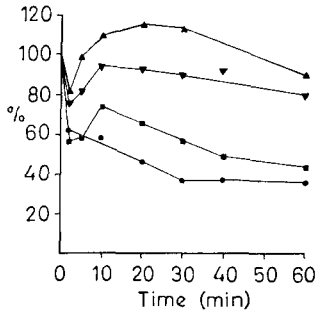

FIG. 3. Dose-response of the effect of strychnine methiodide (μmol each eye) on the amplitude of the b-wave (%) in response to constant stimuli (filter intensity of 3·0 and stimulus duration of 40 ms) as a function of time (min). The amplitude before injection served as 100%. ● Saline; ▼ 0·04; ▲ 0·10; ■ 0·15 μmol strychnine methiodide each eye.

from the trough of the a-wave to the peak of the b-wave as indicated in Fig. 1B. The b-wave amplitude at 0 min served as a control (i.e. 100%). The results are shown in Fig. 2. In the saline-injected rats, the b-wave amplitude dropped considerably 2 min after the injection and then gradually decreased afterward. Strychnine at a low dose (0·06 μmol each eye) maintained the b-wave amplitude around 100%, which is about twice that of the saline-injected control. At a higher dose (0·15 μmol each eye), the b-wave amplitude was diminished within 5 min after injection for about 20 min, then gradually recovered and slightly overshot that of saline control. At an intermediate dose (0·10 μmol each eye), the effect was biphasic, i.e. the b-wave amplitude decreased below that of the saline control within 15 min and then recovered way above the saline control afterward. Therefore, the effect of strychnine is dose-dependent and biphasic.

Strychnine in the uncharged form is lipid-soluble and should cross the membrane readily. Certain local anaesthetics, when converted into quaternary form, are effective in blocking ionic conductances only when applied internally to squid axons (Frazier, Narahashi & Yamada, 1970). The strychnine effect on the ERG (Pong & Graham, 1976) and on the lobster giant axon membrane (Dr A. R. Freeman, personal communication) was similarly abolished by quaternization. The time course effect of quaternary strychnine on the amplitude of the b-wave was studied in a similar fashion and the typical results illustrating the salient features of the effect are shown in Fig. 3. At all doses tested, quaternary strychnine maintained the b-wave amplitude above that of the saline-injected control. The effect was dose-dependent from 0·04 to 0·10 μmol each eye. Beyond the latter dose, however, the effect was attenuated, probably due to being masked by the prominent rhythmic potentials induced at that dose (Pong & Graham, 1976).

In some textbooks related to ophthalmology (e.g., Grant, 1974), one finds an evasive statement about the possible effect of strychnine in amblyopia. Uhthoff & Metzger (1931) noted that there had been claims that small doses of strychnine increased visual acuity and enlarged the visual fields and that on the basis of these claims the drug was much used in the past in the treatment of various visual disturbances. Although the validity of these results had been disputed (Schlaginweit, 1922; Uhthoff & Metzger, 1931; Oskarsson, 1962; Grant, 1974) and strychnine is no longer used clinically in ophthalmology, it does seem that many observers have agreed that the instillation of strychnine into the conjunctival sac increases visual acuity.

The results of this investigation indicate that strychnine either maintained the b-wave amplitude twice that of the control at low dose, or decreased the a-like wave threshold approximately 3 orders of magnitude in response to a low intensity flash stimuli at high dose. The overall effect is to increase the retinal sensitivity in response to light. Based on the present investigation on the effect of strychnine on rat ERG *in vivo*, the early claims of the possible beneficial effect of strychnine in amblyopia is not without substance.

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REFERENCES

CONE, R. A. (1963). *J. gen. Physiol.*, **46**, 1267–1286.
DOWLING, J. E. (1967). *Science*, **155**, 273–279.
FRAZIER, D. T., NARAHASHI, T. & YAMADA, M. (1970). *J. Pharmac. exp. Ther.*, 171, 45–51.
GRAHAM, L. T., Jr. & PONG, S. F. (1972). *Expl. Neurol.*, **36**, 399–403.
GRANT, W. M. (1974). *Toxicology of the Eye*. 2nd edn, pp. 945–946, Springfield, Illinois: Thomas.
OSKARSSON, V. (1962). *Acta Pharmac.*, **19**, 16–22.
PONG, S. F. & GRAHAM, L. T., Jnr. (1976). *Archs int. Pharmacodyn. Thér.*, **220**, 275–286.
SCHLAGINWEIT, E. (1922). *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.*, **95**, 104–123.
UHTHOFF, W. & METZGER, E. (1931). *Handb. d. norm. u. path. physiol.*, **12**, 812–133.