Effects of Ergocornine and Reserpine on Metamorphosis in Bufo bufo japonicus Tadpoles

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Synopsis

Effects of ergocornine and reserpine on tail resorption during metamorphosis in Bufo bufo japonicus tadpoles were studied. At prometamorphosis, ergocornine induced precocious tail resorption, while reserpine scarcely affected the tail size. At the climax of metamorphosis, ergocornine was almost ineffective in accelerating tail resorption, while reserpine was effective in inhibiting tail resorption. Since prolactin-like hormone is known to block the tail resorption induced by thyroid hormones, it was postulated that the release of the hormone is blocked by ergocornine during prometamorphosis and stimulated by reserpine during climax.

Mammalian prolactin has antimetamorphic effects on many amphibian species (Campantico et al., 1964; Nicoll et al., 1965; Etkin and Gona, 1967; Bern et al., 1967). It has also been reported that amphibian pituitary glands contain the antimetamorphic factor(s) (Derby and Etkin, 1968; Yamamato et al., 1979). Eddy and Lipner (1975) has shown that tadpoles have a substance cross-reacting with ovine prolactin antiserum, that blocks the effects of thyroid hormones. Furthermore, we have obtained the result that among the fractions separated electrophoretically from the bullfrog pituitary gland, only the fraction having a prolactin activity blocked thyroxine-induced resorption of the tadpole tail (Kikuyama et al., in press). Therefore, the antimetamorphic hormone is considered to be a prolactin-like hormone.

In mammals, it is well known that the release of prolactin is accelerated by reserpine and inhibited by ergocornine (see MacLeod, 1976). The present experiments were carried out to study the effect of these pharmacological agent on metamorphosis in Bufo bufo japonicus larvae.

Materials and Methods

Eggs of Bufo bufo japonicus were hatched in our laboratory at 23°C. Tadpoles were fed on boiled spinach. The metamorphic process was classified into three stages, prometamorphosis, metamorphosis and climax according to Etkin (1968). When the animals reached the prometamorphic stage, each of 54 tadpoles was transferred to a glass container (10 cm in diameter). Eighteen were injected with 0.01 ml saline. Other 18 were given injections of 10 μg ergocornine (ergocornine maleate, Sandoz) in 0.01 ml saline. The remaining 18 received an injection of 0.01 ml (1 μg) of reserpine (Daiichi Seiyaku) solution. Intraperitoneal injections were performed every day. Tail length and tail height, which were generally used as criteria of metamorphosis, were measured on day 0, 7, 9, 10, 11, and 12. The results were expressed as the percentage change from the initial values. After 12 days of treatment, heads of 5 specimens of control and reserpine treated groups were freeze-dried. To confirm the monoamine-depleting effect of reserpine, hypothalamic monoamine-containing neurons were observed using the histo-
Results

Emergence of front limbs (beginning of climax) occurred 9.4 ± 0.4 (Mean ± S.E.), 9.5 ± 0.4 and 10.8 ± 0.5 days after the first injection in the control, ergocornine-treated and reserpine-treated groups, respectively. The difference between the reserpine-treated and the other two groups was significant (p < 0.05).

In the ergocornine-treated tadpoles, a considerable reduction in the tail height occurred during prometamorphosis for the first 9 days. In the control and reserpine-injected tadpoles, tail height was also gradually reduced at this period but the change was not so drastic as in the ergocornine-treated group (Fig. 1). The tail length remained almost unchanged in the reserpine-injected animals for the first 9 days, while in the control and ergocornine-treated animals, the tail length was shortened slightly for the same period (Fig. 2).

During climax (9–12 days after the first injection), the tail height reduction in the controls became prominent and eventually the tail height value came close to that of the ergocornine-treated group (Fig. 1). The tail length of the control and ergocornine-treated animals was also reduced remarkably (Figs. 2 and 3). In the reserpine-treated tadpoles, both tail height and tail length were reduced more slowly than in the other two groups even after they reached climax (Figs. 1, 2 and 3).

In the reserpine-treated tadpoles less fluorescent neurons in the paraventricular organ (PVO) were observed than in the tadpoles of the control group (Fig. 4, A and B). Neurons in the preoptic recess organ (PRO) of the reserpine-treated emitted less fluorescence than those of the controls.

Discussion

In the present experiments reserpine depleted monoamines in the hypothalamic...
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Fig. 3. Photograph of tadpoles injected with saline (C), ergocornine (E) and reserpine (R) for 12 days. ×2.

Fig. 4. Frontal section of paraventricular organ (PVO) in toad tadpoles. A, saline-injected; B, reserpine-treated; V, third ventricle. ×200.

neurons and retarded tail resorption. On the other hand, ergocornine accelerated tail regression. In mammals, it is well known that reserpine brings about prolactin release by lowering hypothalamic monoamine levels and that ergocornine inhibits prolactin release by its stimulatory effect on dopamine receptors in the pituitary gland (see Macleod, 1976). Therefore, it is probable that the release of prolactin-like hormone, which is known to counteract thyroid hormones to block the tail resorption, was also accelerated by reserpine and inhibited by ergocornine as in the case of mammalian
prolactin. Platt (1976) reported that ergocornine did not enhance the thyroidal function in tiger salmanders. Although there has been no report that reserpine affects the thyroidal activity in amphibians, the tranquilizer is said to have an antithyroid activity in rats (Mayer et al., 1956; Bierwagen and Smith, 1959). Accordingly, the possibility that reserpine retarded tail resorption by lowering thyroid hormone-level could not be excluded.

Recently we have observed that in Rana catesbeiana tadpoles kept in thyroxine solution, reserpine and ergocornine affected the induced metamorphosis as they did the natural metamorphosis in toad tadpoles. This indicates that reserpine and ergocornine were effective in altering the metamorphic speed, even if they did not influence the thyroidal activity. We have also observed that pimozide, a specific blocker of the dopamine receptor in mammals (Jansen et al., 1968; Anden et al., 1970), blocked thyroxine-induced metamorphosis and that it nullified the effect of ergocornine. Furthermore, these pharmacological agents were ineffective in affecting thyroxine-induced metamorphosis in the hypophysectomized specimens (Seki and Kikuyama 1978).

We have demonstrated that monoaminergic neurons in the preoptic recess organ of Bufo bufo japonicus tadpoles develop as metamorphosis proceeds and that the development of these neurons is dependent on thyroid hormones (Kikuyama et al., 1979). In the present experiments, it was revealed that reserpine acted on these neurons as well as the neurons in paraventricular organ to deplete monoamin contents. The fluorescent materials in the preoptic recess organ is presumed to be dopamine (Prasada Rao and Hartwing, 1974). It is probable that these neurons are responsible for the inhibition of prolactin-like hormone during metamorphic climax.

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