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# SHORT COMMUNICATIONS

## Tryptophan pyrrolase induction by hydrosoluble or liposoluble glucocorticoid esters

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LIVER tryptophan-pyrrolase (TPO), an enzyme (E.C.1.13.1.12) which catalyzes the conversion of L-tryptophan to L-formylkynurenine, is sensitive, in the adrenalectomized rat, to induction both by its substrate—tryptophan—and by the glucocorticoids.[1, 2] The degree of the amount of increase in the TPO activity in adrenalectomized rats after administration of the glucocorticoids may be used for the biological assay of the glucocorticoid activity.[3] In the intact animal, the increase in the TPO activity 6–7 hr after the administration of an ACTH-producing drug was utilised as a test of activation of the glucocorticoid adrenal secretion.[4–7]

As part of general investigation of the sp. act. of hydrosoluble or liposoluble corticoid esters we studied the behaviour of the liver TPO in adrenalectomized rats. The animals were treated with a liposoluble ester of hydrocortisone (h.) and of prednisolone (p.) (h. or p. acetate) or hydrosoluble esters (h. or p. sodium succinate, p. sodium phosphate) by the intraperitoneal or the oral route.

### METHODS

Male Wistar rats, weighing 132–186 g were used. Four to five days before experimentation, these animals were adrenalectomized under ether anaestesia using the dorsal approach.

The animals were maintained on a balanced synthetic diet consisting of pellets (ARSAL, Rome) up to 12 hr before the tests. Saline was always accessible for drinking purposes.

The corticoid esters (h. and p. acetate; h. and p. sodium succinate; p. sodium phosphate) were administered to rats kept fasting for 12 hr, but with free access to saline. The steroids were given by the i.p. or by the oral route in fine aqueous suspension (h. or p. acetate) or in solution (h. or p. sodium succinate, p. sodium phosphate) in a volume of liquid not greater than 10 ml/kg.

Adrenalectomized animals treated with saline (10 ml/kg by oral or i.p. route) were used as controls. The corticoid esters were administered at the following doses (as free alcohols): 10 mg/kg body wt. for h. acetate or sodium succinate; 5 mg/kg b.w. for p. acetate, sodium phosphate or sodium succinate. At least 5 rats for each corticoid ester and for each administration route were employed.

The TPO activity was determined in the livers of decapitated animals 6½ hr after the administration of the steroids according to Knox's method[8] and expressed in Units. One Unit is equivalent to 1 $\mu$M of kynurenine formed per g of liver and per hour.[9]

### RESULTS AND DISCUSSION

Values of 1·78 $\pm$ 0·023 TPO Units were found in normal intact rats. These values are significantly higher than those observed in adrenalectomized rats treated with saline (Table 1). Prednisolone as well as hydrocortisone, given as acetates present an intense stimulating activity on the liver TPO. The induction of the enzyme is of the same intensity whether the products are given in the same dose by either the oral or i.p. route. On the other hand, the i.p. injection of equivalent doses of hydrocortisone sodium succinate and prednisolone sodium succinate as well as prednisolone phosphate (all soluble in water), present a stimulating activity on the liver TPO which is clearly inferior to that of the respective liposoluble acetates.

Furthermore, one can observe a clear difference with respect to the induction of TPO by a steroid made hydrosoluble, depending on whether the compound is given orally or by the i.p. route. In the first case, the enzyme-induction is clearly superior to that noted when the hydrosoluble steroid is given by the i.p. route. However, the induction of TPO activity by the orally administered hydrosoluble corticoids (excluding prednisolone sodium succinate), is inferior to that of the same steroids given as acetates.

TABLE 1. LIVER TRYPTOPHAN PYRROLASE IN ADRENALECTOMIZED RATS TREATED WITH HYDROSOLUBLE OR NOT HYDROSOLUBLE CORTICOID ESTERS

| Treatment | Tryptophan pyrrolase Units in rats treated by | | P oral route-i.p. route |
|---|---|---|---|
| | oral route | i.p. route | |
| Saline (Controls) | 1·10 ± 0·21 | 1·04 ± 0·13 | |
| Hydrocrotisone acetate | 6·28 ± 0·64 | 6·55 ± 0·37 | 0·7–0·6 |
| Hydrocortisone sodium succinate | 4·34 ± 1·06 | 2·70 ± 0·17* | 0·2–0·1 |
| Prednisolone acetate | 6·63 ± 0·45 | 7·96 ± 0·77 | 0·2–0·1 |
| Prednisolone sodium succinate | 7·97 ± 0·81 | 2·24 ± 0·16* | <0·01 |
| Prednisolone sodium phosphate | 4·64 ± 1·09 | 2·30 ± 0·16* | 0·05–0·02 |

All differences are statistically significant ($P<0·01$) with respect to the controls.
* $P<0·01$ with respect to the values found in the rats treated with the same steroid given as acetate.

In other research work,[10] we have noted thymolytic and anti-inflammatory activity of hydrocortisone and prednisolone. This activity is clearly inferior when the above mentioned steroids are given as hydrosoluble esters by the parenteral routes than when they are administered as liposoluble esters (such as acetates).

A similar phenomenon was noted in the rat by Terragna and Jannuzzi[11] with regard to the capacity of prednisolone given intramuscularly to inhibit the formation of hemolysins, and by Jequier *et al*[12] with regard to the anti-inflammatory activity of the subcutaneous administration of prednisolone and one of its hydrosoluble preparations.

Some significant pharmacokinetic differences (e.g. more rapid absorption and excretion)[13] may account for the weaker activity of hydrosoluble corticoid esters with respect to the liposoluble ones when injected by the intramuscular or s.c. route. The hydrolysis which both hydrosoluble and liposoluble esters undergo in the intestinal tract may be responsible for a more uniform absorption and efficiency when these esters are given by gastric tubage.

Studies currently in progress in this Laboratory clearly demonstrate that the observed differences concerning the TPO induction by corticoid esters given by different administration routes may be related to the relative speed of absorption and excretion of these steroids *in vivo*.

On the other hand, the diminished glucocorticoid activity of s.c. or intramuscular administration of hydrosoluble corticoid esters seems important when one considers that, in man, the intramuscular and i.v. routes are considered preferable for the administration of the hydrosoluble steroids mentioned above. For instance, some of these, when injected s.c. (hydrocortisone sodium succinate,[14] prednisolone sodium succinate)[15] or i.v. (dexamethasone-21-phosphate)[15] were found weakly effective or completely ineffective in the guinea pig's anaphylactic shock.

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## Receptors of neurotransmitters—III. Comparison of the 5-hydroxytryptamine receptor of the liver fluke, *Fasciola hepatica*, and the rat stomach-fundus

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EARLIER investigations on smooth muscle indicated a relationship between the receptor function for 5-HT* and sialic acid metabolism.[1] Different *N*-acyl-neuraminic acids and gangliosides increased sensitivity as well as maximal height of 5-HT induced contraction of the rat stomach fundus while synthetic inhibitors of NANA-biosynthesis decreased the contraction. In an attempt to get further information about the 5-HT receptor by comparing receptors from different species, we studied the influence of sialic acid metabolism and of adamantanamines on the 5-HT induced activity of the liver fluke, *Fasciola hepatica*. The adamantanamines were found to give an increased response of the rat fundus strip to 5-HT[2].

According to Welsh[3] 5-HT may play a role as mediator in nerve action in certain invertebrates. Erspamer[4] found 5-HT and other biological active indolamines in many tissues of invertebrates.

Liver flukes were collected from bovine livers within 1 hr after death of the host and kept in buffered (dH 8·5) Ringer's solution at 37° as described by Chance and Mansour.[5] Kymograph registrograms were made within 24 hr after dissecting the flukes. The fluke was incubated in 10 ml Ringer's solution at 37°. Oxygen was passed through the vessel to facilitate the distribution of added compounds. The fluke being under slight tension[5] was fixed to one end of an isotonic level giving about 5 times magnification and allowed 10 min to acclimatize before starting with experiments. Each contraction was registered for 5 min and the drug was washed out for 10 min before the following contraction was produced. Thus one preparation could be used for 90–120 min.

Using $5 \times 10^{-4}$ M 5-HT or amphetamine sulphate as an agonist the contraction height of the fluke can be maintained constant for at least 90 min (Fig. 1). Mansour[6] suggested that amphetamine and

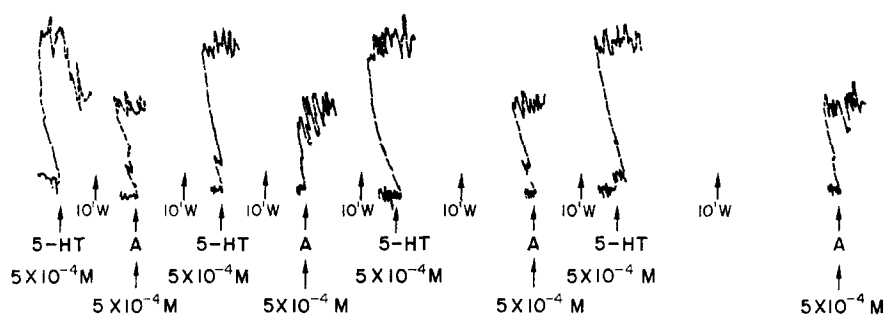


FIG. 1. Kymograph registrogram of 5-HT and amphetamine sulphate (A) induced contraction on the liver fluke, concentration $5 \times 10^{-4}$ M.

* Abbreviations used: 5-HT = 5-hydroxytryptamine (serotonin); NANA = *N*-acetylneuraminic acid; NANA-9-P = *N*-acetylneuraminic acid-9- phosphate.