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Possible oestrogenic and anti-androgenic effects in rats treated with the oral hypoglycaemic agents tolbutamide and acetohexamide

SAMIR FAHMY SAAD

Department of Pharmacology, Faculty of Pharmacy, Cairo University, Cairo, U.A.R.

Tolbutamide has an oestrogenic activity; when administered in four oral daily doses of 125, 250 and 500 mg/kg to ovariectomized young rats, it significantly increased their uterine weight. Tolbutamide, when administered in five oral daily doses to castrated young male rats, decreased the weight of the prostatic tissue; the effect was significant with a dose of 250 mg/kg. Acetohexamide in oral doses of 125, 250 and 500 mg/kg, had no significant effect either on the prostatic or the uterine weight.

Since the discovery of the oral hypoglycaemic agents, much work has been done to investigate their effects on different endocrine systems especially those concerned with the regulation of carbohydrate metabolism. No marked attention has been paid to their effects on sex hormones.

The effect of tolbutamide and acetohexamide on the activities of the gonadal hormones has been examined.

EXPERIMENTAL AND RESULTS

Determination of oestrogenic activity

The activity was determined as described by Bülbring & Burn (1935) using the increase in the uterine weights of ovariectomized rats as a criterion.

Thirty-five young female rats, each weighing about 40 g, were ovariectomized. Two days later, the animals were divided into 7 equal groups. One group, used as control, received no drug. Tolbutamide and acetohexamide were given orally in doses of 125, 250 and 500 mg/kg to respective groups, each dose being administered daily for 4 successive days, the animals were then left for a day, and killed on the following day. Thus if the day of the operation was the first day, drugs were given on the third, fourth, fifth and sixth days, and the animals were killed on the eighth day. The dissected uteri were fixed separately in modified Bouin's solution for 24 h, dried between pieces of filter paper and weighed. The weight of uterus per 100 g of rat was calculated. The results are in Table 1.

Determination of anti-androgenic activity

The activity was determined as described by Burn, Finney & Goodwin (1950). The changes in weight of the prostate and seminal vesicles of castrate rats were taken as criteria. Young male rats were used because, according to Sollmann (1957), they are more sensitive to the effect of oestrogens.

Table 1. *The effect of four oral daily doses of tolbutamide and acetohexamide on uterine weights of ovariectomized young rats compared with controls*

Sample number and statistical data	Weight of uterus (mg/100 g)						
	Controls	Tolbutamide (mg/kg)			Acetohexamide (mg/kg)		
		125	250	500	125	250	500
1	40·0	45·3	66·7	50·2	36·8	38·0	40·6
2	42·4	60·2	70·0	60·0	39·0	40·2	43·7
3	44·0	71·0	78·4	78·9	48·6	45·4	48·9
4	57·0	80·3	90·0	82·5	57·5	60·2	58·5
5	58·2	82·5	92·0	108·7	60·0	65·3	68·9
$\bar{x}$	48·32	67·86	79·42	76·06	48·38	49·82	52·12
± s.e.	3·8459	6·8797	5·1077	10·1050	4·6925	5·4745	5·1798
*P**		s.	s.	s.	n.s.	n.s.	n.s.

* The difference between the mean weight of uteri after treatment, and the mean of the control weight is considered significant if $P = <0{\cdot}05$.

Thirty-five young male rats, each weighing from 30 to 40 g, were castrated, left for 30 days before the experiment, and then divided into 7 equal groups. One group, used as control, received no drug. Tolbutamide and acetohexamide were given orally in doses of 125, 250 and 500 mg/kg, each dose being administered daily for 5 days. The animals were killed on the sixth day, and the seminal vesicles and prostatic tissue dissected. The tissue consists of the cranial lobes that are attached to the seminal vesicles and more specifically the dorso-lateral lobes around the base of the seminal vesicles and almost encircling the urethra. Particular care was taken not to remove the ventral lobes. The organs of each rat were individually fixed overnight in modified Bouin's solution after removing fat. The tissues were dried between pieces of filter paper and weighed. The weight of each prostatic tissue was calculated in mg/100 g of rat. The results are in Table 2.

Table 2. *The effect of five oral daily doses of tolbutamide and acetohexamide on the weight of prostatic tissue of* 30 *day castrated young rats compared with the controls*

Sample number and statistical data	Weight of prostatic tissue (mg/100 g)						
	Controls	Tolbutamide (mg/kg)			Acetohexamide (mg/kg)		
		125	250	500	125	250	500
1	17·5	14·1	13·6	16·3	15·0	15·1	18·8
2	19·5	15·2	15·5	16·4	17·9	20·6	19·9
3	22·1	18·5	17·5	17·1	18·8	24·0	21·2
4	22·4	19·5	20·2	20·4	20·2	24·0	21·8
5	27·8	23·9	21·3	22·6	21·8	25·0	22·9
$\bar{x}$	21·86	18·24	17·62	18·56	18·70	21·74	20·92
± s.e.	1·7352	1·7330	1·4290	1·2574	1·1367	1·8198	0·7179
*P**		n.s.	s.	n.s.	n.s.	n.s.	n.s.

* The difference between the mean weight of prostatic tissue after a certain treatment, and the mean of the control weight is considered significant if $P = <0{\cdot}05$.

DISCUSSION

Because of the wide and prolonged use of tolbutamide and acetohexamide in the treatment of certain types of diabetes, it is of importance to determine whether or not they possess oestrogenic and anti-androgenic effects.

Tolbutamide was given to ovariectomized and castrated young rats in oral doses of 125, 250 and 500 mg/kg. These doses have an effective hypoglycaemic action in rats, and were non-toxic. Mukherjee & De (1958) found that oral administration of tolbutamide in doses of 250, 500 and 1000 mg/kg to rats lowered their blood sugar content. The maximal hypoglycaemic effect was produced by a dose of 500 mg/kg body weight. The oral LD50 of tolbutamide in rats was determined by Scholz & Bänder (1956) to be 4 g/kg, and by Penhos (1957) to be 4–5 g/kg body weight. The oral LD100 of tolbutamide in rats was determined by Penhos (1957) to be 6 g/kg body weight.

During tolbutamide and acetohexamide administrations and at the end of the experiments, the rats were normal in behaviour, not excited or depressed. Their body weight during the period of drug administration did not vary more than $\pm$ 3 g. The prostatic tissue weight and uterine weight were expressed as proportions of body weight to compensate for any difference in animal's body weight.

The results of Table 1 show that tolbutamide when administered orally in doses of 125, 250 and 500 mg/kg body weight to ovariectomized young rats for 4 successive days produced a significant increase in the mean uterine weight compared with the control. This suggests an oestrogenic activity for tolbutamide.

The results in Table 2 show that tolbutamide when administered orally for 5 successive days in doses of 125, 250 and 500 mg/kg to 30 day castrated young rats, reduced the weight of prostatic tissue compared with the control. The effect was significant with a daily oral dose of 250 mg/kg body weight. This points to a possible anti-androgenic effect for tolbutamide, although the decrease in the mean weight of prostatic tissue of castrated young rats may reflect the oestrogenic activity of tolbutamide. According to Sollmann (1957), continued administration of oestrogens produces atrophy of the accessory sex organs including the seminal vesicles in immature animals.

Acetohexamide, as compared with tolbutamide, when similarly tested in ovariectomized and castrated young rats had neither an oestrogenic nor an anti-androgenic activity. This suggests that the actions of tolbutamide on uterine and prostatic tissue is not due to its hypoglycaemic action, but instead reflects a specific secondary pharmacological action.

These hormonal effects of tolbutamide would seem to need clinical evaluation.

REFERENCES

BÜLBRING, E. & BURN, J. H. (1935). *J. Physiol., Lond.*, **85**, 320–333.

BURN, J. H., FINNEY, D. J. & GOODWIN, L. G. (1950). *Biological Standardization*, 2nd edn, p. 262, Oxford: University Press.

MUKHERJEE, S. K. & DE U. N. (1958). *Indian J. med. res.*, **46**, 223–233.

PENHOS, J. C. (1957). *Rev. Soc. Arg. biol.*, **33**, 44–50.

SCHOLZ, J. & BÄNDER, A. B. (1956). *Dt. med. Wschr.*, **81**, 825–826.

SOLLMANN, T. (1957). *A Mannual of Pharmacology and its Application to Therapeutics and Toxicology*, 8th edn, p. 615. Philadelphia: Saunders.