Effect of diazepam on plasma corticosterone levels in the rat

The activity of steroid hormones may be altered by many drugs not necessarily chemically or pharmacologically related. Among sedatives and tranquillizers, the effect of barbiturates, morphine, ethanol, reserpine, chlorpromazine and meprobamate on the pituitary adrenal axis is well known (Gaunt, Chart & Renzi, 1965; Gaunt, Steinetz & Chart, 1968; Kakihana, Noble & Butte, 1968).

Little is known about the action of benzodiazepines on steroid activity and what is known seems contradictory. According to Dasgupta & Mukherjee (1967a) chlordiazepoxide in the rabbit shows an inhibitory action on the eosinopenia induced by stress or by ACTH. In addition it seems to have a protective action against stress-induced stomach ulcers (Dasgupta & Mukherjee, 1967b). Superstine & Sullman (1966) noticed an increased 17-ketosteroid excretion and an increased adrenal weight after chronic administration of chlordiazepoxide and diazepam in mice. Butler, Besser & Steinberg (1968) described a marked fall of plasma cortisol levels after chlordiazepoxide administration in man. Because the experimental evidence suggests a hypothalamic action (Shallek & Zabransky, 1966) we report the influence of small doses of diazepam on plasma corticosterone concentration.

Male Sprague-Dawley rats (150–200 g), housed in standardized conditions (22° and 60% humidity), were used. All the experiments were made in the morning. The rats, housed singly in cages and put in an acoustically isolated room 16 h before the experiment, received diazepam at 0·5; 1; 2·5; 5 and 10 mg/kg by intraperitoneal or oral route as indicated in Table 1.

Table 1. *Plasma concentrations of corticosterone* (μg/100 ml) *in the rat after a single treatment of diazepam*

Dose mg/kg	1 h (a)	2 h (b)
Solvent	6·7 ± 0·55	5·2 ± 0·4
Diazepam 0·5 i.p.	5·0 ± 0·4	5·0 ± 0·4
Diazepam 1 i.p.	7·6 ± 0·9	6·2 ± 0·4
Diazepam 2·5 i.p.	17·4 ± 1·6†	16·0 ± 1·5*
Diazepam 5 i.p.	49·6 ± 2·4†	12·1 ± 1·3*
Solvent, orally	9·4 ± 0·7	6·3 ± 0·6
Diazepam 5 orally	10·0 ± 1·7	11·0 ± 3·0
Diazepam 10 orally	12·0 ± 2·7	35·0 ± 7·2*

a = 6–10 animals, each group.
b = 6 animals, each group.
* P <0·01 with respect to solvent group.
† P <0·001 with respect to solvent group.

Blood samples were collected after 1 and 2 h and tested for corticosterone (Guillemin, Clayton & others, 1959). Both controls and diazepam-treated animals show an immediate increase of plasma corticosterone levels within 5–15 min from the injection. This effect, due to the handling, disappeared within 1 h. At 60 min the effect of the drug is clearly evident. As summarized in Table 1, plasma concentrations of corticosterone are significantly higher in the animals treated with 2·5 and 5 mg/kg intraperitoneally and remain so for 2 h, after which corticosterone concentrations approach the control values. The lower doses (0·5 and 1 mg/kg, i.p.) had no effect. After oral administration, the increase of corticosterone appears after 2 h but only with higher dosages (10 mg/kg). After repeated treatment the high corticosterone concentration is still present; either after the three days or after the eight day treatment, the corticosterone concentrations are higher than control values 60 min after the last administration of diazepam (Table 2).

Table 2. *Plasma levels of corticosterone* (μg/100 ml) *in the rat after repeated diazepam administration*

Dose mg/kg	1 h (a)	2 h (a)
Solvent, i.p. thrice daily for 3 days	7·59 ± 0·98	1·95 ± 0·28
Diazepam 1, i.p. thrice daily for 3 days	5·60 ± 0·76	2·70 ± 1·34
Diazepam 2·5, i.p. thrice daily for 3 days	15·75 ± 1·79†	4·19 ± 1·2*
Solvent, i.p. thrice daily for 8 days	9·73 ± 1·45	—
Diazepam 0·5, i.p. thrice daily for 8 days	6·75 ± 0·98	—
Diazepam 1, i.p. thrice daily for 8 days	6·36 ± 0·43	—
Diazepam 2·5, i.p. thrice daily for 8 days	31·48 ± 2·01†	—

a = 6 animals, each group.
* $P < 0.01$ with respect to solvent group.
† $P < 0.05$ with respect to solvent group.

The effect seems to be mediated, as for other drugs, through ACTH release via the hypothalamus and pituitary. A direct action on the adrenals does not appear to be present since after dexamethasone pretreatment (0·2 mg/kg 4 h before diazepam) there is no response to diazepam. Moreover, in our experimental conditions, diazepam seems to be differentiated from chlordiazepoxide. In fact the fall in corticosterone concentrations, as described for chlordiazepoxide (Dasgupta & Mukherjee, 1967a,b; Butler, Besser & Steinberg, 1968) was not present. In addition, the repeated treatment with diazepam resulted in a persistent increase of plasma corticosterone differing from that described for reserpine (Wells, Briggs & Munson, 1956; Kitay, Holub & Jailer, 1959; Maickel, Westermann & Brodie, 1961; Khazan, Sulman & Winnik, 1961; Gaunt, Chart & Renzi, 1965), chlorpromazine (Gaunt & others, 1968) and meprobamate (Gold, Di Raimondo & others, 1960; Mäkelä, Näätänen & Rinne, 1959). Recently Jori, Prestini & Pugliatti (1969) described an inducing effect of diazepam on liver metabolic activities, when given chronically at high dosages. The above reported increase in corticosterone plasma concentrations could partially be involved with this inducing effect of the drug. Metabolic activities are increased under stress (Driever, Bousquet & Miya, 1966) and on the other hand the inducing effect of steroids on hepatic microsomal enzymes has been reported (Gelboin & Conney, 1968). Recently a close relation between liver enzymatic activities and pituitary adrenal function has also been described (Orrenius & Ernster, 1967; Radzialowsky & Bousquet, 1968).

This work was financially supported by Contract DHEW/PHS.NIH/PH 43-67-83.

Istituto di Ricerche Farmacologiche "Mario Negri",
Via Eritrea, 62, 20157 *Milan, Italy.*

V. MARC
P. L. MORSELLI*

July 10, 1969

* Merck, Sharp & Dohme Fellow.

REFERENCES

BUTLER, P. W. P., BESSER, G. M. & STEINBERG, H. (1968). *J. Endocr.*, **40**, 391–392.

DASGUPTA, S. R. & MUKHERJEE, B. P. (1967a). *Nature, Lond.*, **213**, 199–200.

DASGUPTA, S. R. & MUKHERJEE, B. P. (1967b). *Ibid.*, **215**, 1183.

DRIEVER, C., BOUSQUET, W. F. & MIYA, T. S. (1966). *J. Neuropharmac.*, **4**, 199–205.

GAUNT, R., CHART, J. J. & RENZI, A. A. (1965). *Ergebn. Physiol.*, **56**, 114–172.

GAUNT, R., STEINETZ, B. G. & CHART, J. J. (1968). *Clin. Pharmac. Ther.*, **9**, 657–681.

GELBOIN, H. V. & CONNEY, A. H. (1968). In "*Modern trends in toxicology*". Editors: Boyland, E. & Goulding, R., pp. 175–197. London: Butterworth.

GOLD, E. M., DI RAIMONDO, V. C., KENT. J. R. & FORSHAM, P. H. (1960). *Ann. N.Y. Acad. Sci.*, **86**, 178–190.

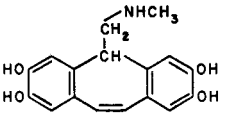
GUILLEMIN, R., CLAYTON, G. W., LIPSCOMB, H. S. & SMITH, J. D. (1959). *J. Lab. clin. Med.*, **53**, 830–832.

JORI, A., PRESTINI, P. E. & PUGLIATTI, C. (1969). *J. Pharm. Pharmac.*, **21**, 387–390.
KAKIHANA, R., NOBLE, E. P. & BUTTE, J. C. (1968). *Nature, Lond.*, **218**, 360–361.
KHAZAN, N., SULMAN, F. G. & WINNIK, H. Z. (1961). *Proc. Soc. exp. Biol. Med.*, **106**, 579–581.
KITAY, J. I., HOLUB, D. A. & JAILER, J. W. (1959). *Endocrinology*, **65**, 548–554.
MAICKEL, R. P., WESTERMANN, E. O. & BRODIE, B. B. (1961). *J. Pharmac. exp. Ther.*, **134**, 167–175.
MÄKELÄ, S., NÄÄTÄNEN, E. & RINNE, U. K. (1959). *Acta Endocr.*, **32**, 1–7.
ORRENIUS, S. & ERNSTER, L. (1967). *Life Sci.*, **4**, 1473–1482.
RADZIALOWSKI, F. M. & BOUSQUET, W. F. (1968). *J. Pharmac. exp. Ther.*, **163**, 229–238.
SHALLEK, W. & ZABRANSKY, F. (1966). *Archs int. Pharmacodyn. Thér.*, **161**, 126–131.
SUPERSTINE, E. & SULLMAN, F. G. (1966). *Ibid.*, **160**, 133–146.
WELLS, H., BRIGGS, F. N. & MUNSON, P. L. (1956). *Endocrinology*, **59**, 571–579.

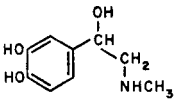
An improved method for the preparation of adnamine

We have recently been interested in the preparation of some *N*-alkyl substituted homologues of noradnamine (5-aminomethyl-2,3,7,8-tetrahydroxydibenzo[*a*,*e*]cycloheptatriene) for pharmacological testing. The *N*-methyl derivative, adnamine (I), was originally obtained by Kawazu (1958) by boiling a solution of adrenaline (II) in 10% hydrochloric acid for 3·5 h. Recently Roberts & Broadley (1967) reported that the main crystalline product usually obtained in this manner was not adnamine (I) but was identical to the product isolated previously by Funk & Freedman (1923) and Öppinger & Vetter (1942) and described as diadrenaline ether (III). Roberts & Broadley (1967) further reported that reasonable yields of adnamine (I) could consistently be obtained with stronger acids and longer reaction times than those employed by Kawazu. The production of adnamine (I) by this latter procedure, is, however, often accompanied by the copious formation of tarry by-products which complicates the isolation and purification of the product.

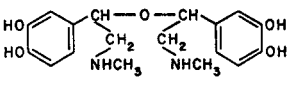
Recent work in these laboratories has shown that the product previously described as diadrenaline ether (III) does not have structure III but is, in fact, 6-(3′,4′-dihydroxy-α-methylaminomethylbenzyl)adrenaline (IV). The trivial name adrepine was proposed for this substance (Forrest, Kašpárek & others, 1969). Preliminary paper chromatographic evidence suggested that IV was an intermediate in the conversion



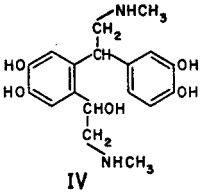
I



II



III



IV