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SHORT COMMUNICATIONS

Adrenocortical and enzymic effects of imipramine and chlorpromazine*

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THE pituitary–adrenocortical system is affected by anesthetics,[1] barbiturates,[2] narcotics,[3] and tranquilizers,[4] but in many cases the nature of these affects is unclear. In particular, the literature on tranquilizers seems contradictory regarding the conditions eliciting adrenocortical response and the relationship between hormonal changes and clinical properties of the drugs.[5–7]

Because of the many chemical and pharmacological similarities of chlorpromazine, a tranquilizer, and impramine, an antidepressant, it seemed of interest to compare the adrenocortical affects of these agents.

MATERIALS AND METHODS

Procedures were carried out on adult male Sprague-Dawley rats, weighing between 120 and 350 g, maintained in individual cages. On the day preceding experimental manipulations nine animals were weighed and divided into three weight-matched treatment groups. Experimental animals received an i.p. injection of 25 mg chlorpromazine† or imipramine‡ per kg at 5 : 00 p.m. and a second i.p. injection of 50 mg/kg at 6.30 the following morning. Controls were injected with saline on both occasions, the injection volume being equal to the largest volume given either of its experimental pairs. The animals were decapitated 3·5 hr after the morning injection, trunk blood collected, and liver and adrenals quickly dissected, weighed, and placed in appropriate media for analysis. In a parallel experiment the second injection was omitted.

Livers were analyzed for tryptophan and phenylalanine hydroxylases[8] and α-ketoglutarate-tyrosine and -tryptophan transaminases.[9] Adrenals were analyzed for ascorbic acid;[10] corticosterone was determined in adrenals and serum.[11] Protein was determined by u.v. absorption,[12] and specific enzyme activities were calculated on this basis. The homogenizing medium was 0·15 M KCl for all enzyme assays.

The order of injection, sacrifice, and analysis was rotated across treatment groups and differed for each block. The data were examined by analysis of variance for a randomized block design, and statistical significance between groups was determined by Duncan's New Multiple Range Test.[13]

RESULTS AND DISCUSSION

The classical indices of adrenocortical stimulation are increased levels of adrenal and blood corticoids and decreased adrenal ascorbic acid. After a single stimulation, adrenal ascorbic acid descends to a nadir in 1 to 2 hr, slowly rises after 4 hr, and returns to baseline within 8 to 12 hr.[14] Corticoids abruptly increase to a maximum in 15 to 30 min and drop to baseline within 1 to 2 hr.[15]

The data in Table 1 show that both imipramine and chlorpromazine elicited these indices of adrenocortical activation. The magnitude of the ascorbic acid response and the persistence of a difference in corticoid levels 17 hr after injection of a single dose of chlorpromazine, or 3·5 hr after a second injection, suggests sustained adrenal stimulation (Table 2).

Enzymic concomitants of adrenocortical activation are based on the classic demonstration by Knox[16] of increased tryptophan pyrrolase activity after administration of pharmacologic doses of adrenocorticoids. Among these corticoid-influenced enzymes are liver tryptophan and tyrosine transaminase[17] and tryptophan hydroxylase.[18] Such enzymic indices must be used with caution in assessing "stress," however, since several recent studies[19, 20] have indicated that diverse stresses

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† Thorazine solution supplied by Smith, Kline & French in multiple-dose vial.

‡ Tofranil solution supplied by Geigy in sealed ampules.

elicit diverse patterns of enzymic change. In the present study tryptophan and tyrosine transaminase activity were significantly elevated over control levels by both imipramine and chlorpromazine. These two enzymic activities are thought to be due to a single enzyme,[21] and increased transaminase activity has been reported to follow administration of adrenocorticoids.[17] Activities of phenylalanine and tyrosine hydroxylase were unaffected by either drug.

TABLE 1. ADRENOCORTICAL AND ENZYMIC AFFECTS OF DOUBLE ADMINISTRATION OF IMIPRAMINE AND CHLORPROMAZINE*

	Saline	Imipramine	Chlorpromazine	Relationship	P†
Adrenal					
Weight (mg)	41·0 ± 10·5	43·0 ± 10·7	38·5 ± 8·6	NS¶	
Corticosterone (μg/g)	7·71 ± 2·95	12·42 ± 6·56	24·04 ± 7·44	cpz > imi > sal	0·05
Ascorbic acid (mg/100 g)	390·6 ± 37·9	300·4 ± 54·0	261·7 ± 4·42	cpz > imi > sal	0·005
Serum corticosterone (μg/100 ml)	8·20 ± 4·13	19·22 ± 11·58	19·07 ± 4·37	cpz = imi > sal	0·001
Liver transaminase (μmoles/min/g protein)					
Tyrosine–α-ketoglutarate‡	0·86 ± 0·53	2·92 ± 2·88	5·70 ± 3·02	cpz > imi > sal	0·005
Tyrosine–α-ketoglutarate§	4·86 ± 1·83	12·23 ± 3·91	17·74 ± 5·14	cpz > imi > sal	0·001
Tryptophan–α-ketoglutarate‡	0·071 ± 0·014	0·101 ± 0·044	0·235 ± 0·048	cpz > imi = sal	0·001
Tryptophan–α-ketoglutarate§	0·077 ± 0·016	0·146 ± 0·092	0·310 ± 0·096	cpz > imi > sal	0·005
Liver hydroxylase (μmoles/hr/g protein)					
Phenylalanine	49·7 ± 11·1	49·0 ± 17·8	50·6 ± 5·2	NS	
Tryptophan	4·08 ± 1·38	2·93 ± 0·787	3·52 ± 1·15	NS	

* Animals were given initial i.p. injection of 25 mg drug/kg, injected 13 hr later with 50 mg/kg, and sacrificed 17 hr after initial injection. Animals matched for weight across the three treatment groups, and each value represents the mean for 9 animals ± S.D.

† The smallest P value for the indicated relationship.

‡ Without addition of pyridoxal phosphate.

§ With addition of pyridoxal phosphate.

¶ No significant difference between groups.

TABLE 2. ADRENOCORTICOL AND ENZYMIC AFFECTS OF A SINGLE CHLORPROMAZINE INJECTION*

	Saline	Chlorpromazine	P
Adrenal corticosterone (μg/g)	6·77 ± 1·06	23·83 ± 5·25	0·005
Ascorbic acid (mg/100 g)	412·2	310·0	
Liver transaminase (μmoles/min/g protein)			
Tyrosine–α-ketoglutarate†	1·45 ± 0·54	6·01 ± 3·48	0·025
Tyrosine–α-ketoglutarate‡	5·48 ± 1·85	12·38 ± 1·14	0·005
Tryptophan–α-ketoglutarate†	0·069 ± 0·037	0·243 ± 0·0214	0·005
Tryptophan–α-ketoglutarate‡	0·067 ± 0·037	0·296 ± 0·0535	0·005

* Animal sacrificed 17 hr after injection (i.p.) of 25 mg chlorpromazine/kg body weight. Values are means ± S.D.

† Without addition of pyridoxal phosphate.

‡ With addition of pyridoxal phosphate.

These results support the contention that moderate levels of chlorpromazine elicit adrenocortical activation and demonstrate that such activation is accompanied by significant increases in liver tryptophan and tyrosine transaminase activity. Moreover, these effects are also produced by imipramine, suggesting that this endocrine response is more likely related to other pharmacologic features than those responsible for the clinical usefulness of these drugs. Although there is evidence for a relationship between the sedative and adrenocortical-activating properties of chlorpromazine and reserpine, such a relationship did not appear to exist for imipramine. Beyond a decrease in activity immediately after administration of imipramine, animals seemed to exhibit normal or, indeed, heightened activity later. Under the conditions of this experiment, then, a phenothiazine antidepressant can elicit adrenocortical activation in the same manner as its tranquilizing congener.

Finally, it is unlikely that the discordant results in the literature on chlorpromazine activation of the adrenal cortex can be due to differences in the absolute quantity of drug administered. Over the wide range of body weights employed in this study there appeared to be no correlation between absolute quantity of administered drug and any of these indices of adrenocortical activation.

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Catecholamine biosynthesis *in vivo*: An application of thin-layer chromatography*

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ALTHOUGH thin-layer chromatography has been used previously in the separation of epinephrine and norepinephrine[1, 2] and also of various epinephrine metabolites[3, 4] the application of this technique to the separation of norepinephrine and its biosynthetic precursors, dopa and dopamine, has

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