Control of Catecholamine Release and Degradation by the Glucocorticoids

The important role of glucocorticoids for adrenaline biosynthesis is well established by the extensive studies of WURTMAN and AXELROD[1,2]. The presence of these hormones induces the activity of enzyme phenylethanolamine-N-methyl transferase (PNMT) in normal animals[3,4]. The inactivation of the adrenal cortex by hypophysectomy follows a marked reduction in adrenal PNMT activity[2,3]. In our recent study we found that the glucocorticoids act as a rate limiting factor for the enzyme of catecholamine degradation (monoamine oxidase)[5] in normal rats. The present investigation was performed to see the possible role of glucocorticoids on catecholamine release and urinary excretion. Metopirone, a potent inhibitor of 11-β-hydroxylase in the biosynthesis of corticosteroids[6-9], was utilized as a tool to block the biosynthesis of these hormones. We found that the inhibition of glucocorticoid hormones completely changes the pattern of urinary excretion of free adrenaline and noradrenaline in normal rats. The blood catecholamines, plasmatic glucose and hepatic glycogen are also highly affected. The experiments about the association of glucocorticoids in catecholamine metabolic degradation were also performed by measuring urinary excretion of vanylmandelic acid (VMA) and the activities of enzymes monoamine oxidase (MAO) and catechol-*O*-methyl transferase (COMT) in the adrenal gland.

Materials and methods. Male albino rats weighing 250 $\pm$ 25 g were utilized in all the experiments. The age of the rats ranged from 12 to 13 weeks and they were housed at a constant temperature of 22 °C with 12 h dark and light exposures. The rats were adrenalectomized or hypophysectomized under ether anaesthesia. The adrenalectomized rats were maintained on 0.9% saline for drinking and regular commercial laboratory food. The hypophysectomized rats received 5% sucrose water for drinking for 3 weeks after the operation. The adrenalectomized and hypophysectomized rats were utilized for experiments 3 and 4 weeks after the operation, respectively. The urine was collected in acid washed glass metabolic cages for the period of 24 h by modification[10] of the technique of EULER and LISHAJKO[11]. The normal rats were administered with 50 mg of Metopirone R (Ciba) i.p. just before placing in metabolic cages for 24 h collection of urine. The hypophysectomized rats were injected only once with 4 IU of ACTH Retard (Endopancrine) at the time they were placed in metabolic cages. For tissue and plasma studies separate groups of rats received 50 mg of Metopirone i.p. 20 ml of heparinized blood from 5 rats was used for single determination of adrenaline. 4 ml blood was withdrawn from aorta of each rat under light ether anaesthesia and pooled in a centrifuge tube containing 4 ml 2% sodium fluoride and 3% sodium thiosulfate mixture[12]. The tissues for enzyme and glycogen studies were immediately excised in chilled 0.9% KCl and 60% KOH respectively. Catecholamines in blood and urine were isolated by column chromatography using acid-activated aluminium oxide[13]. Adrenaline and noradrenaline were assayed differentially by spectrofluorometric methods[14] utilizing trihydroxindole reaction. Urinary VMA was measured spectrophotometrically[15] by conversion of VMA to vanillin[16-18]. Hepatic glycogen was assayed by KOH hydrolysis and conversion to glucose[19]. Plasmatic glucose was measured by the technique of HUGGETT and NIXON[20] utilizing glucose oxidase (Biotrol). Enzymes MAO and COMT in the adrenals were assayed by radiometric methods using ^{14}C-tryptamine and ^{14}C-S-adenosyl methionine respectively[21,22]. The enzyme activities are directly expressed in disintegrations per min (dpm) as the dpmextracted represented linear proportionality between μmoles of product extracted and enzyme activity. The statistical analysis were performed by FISHER's Student *t*-test. The mean values are expressed with standard error of the Mean.

Results. Table I indicates the effects of glucocorticoid inhibition by metopirone administration on urinary excretion of adrenaline and noradrenaline. The mean values $\pm$ SEM express the urinary excretion of these two catecholamines/kg body wt./24 h. The percentage ratios of adrenaline and noradrenaline to total amount of adrenaline + noradrenaline are also calculated to show the shifts in pattern of urinary excretion during metopirone treatment to normal and adrenalectomized rats. One injection of 50 mg Metopirone to normal rats produced significant shifts in adrenaline and noradrenaline excretion. 24 h after single injection of metopirone adrenaline urinary excretion rose by 80% while noradrenaline declined by 63% of their control values. Metopirone administration to adrenalectomized rats did not change noradrenaline excretion but adrenaline in urine rose significantly ($P < 0.001$). There were highly significant rises in blood adrenaline during few hours of metopirone administration to normal rats ($P < 0.001$). At 1.5, 2 and 3 h adrenaline in blood rose by 40, 60 and 400% from control levels respectively. The similar treatment to adrenalectomized rats failed to produce any rise of statistical significance in blood adrenaline. The mean values for plasmatic glucose became 143, 200 and 204% of their control levels in normal rats after 15 min, 1 h and 2 h of metopirone administration. The same dose of metopirone under similar circumstances to adrenalectomized rats did not produce any change in blood glycemia from their control values. The rises in glycemia observed in control rats were followed by fall in hepatic glycogen as it declined by 35, 58, 82 and 99% at 30, 60, 110 and 120 min after administration of metopirone.

[1] R. J. WURTMAN and J. AXELROD, Science *150*, 1464 (1965).
[2] R. J. WURTMAN and J. AXELROD, J. biol. Chem. *241*, 2301 (1966).
[3] R. J. WURTMAN and J. AXELROD, Endocrinology *82*, 585 (1968).
[4] F. MARGOLIS, J. ROFFI and A. JOST, Science *154*, 275 (1966).
[5] H. PARVEZ and S. PARVEZ, C. r. Acad. Sci, Paris *274*, 919 (1972).
[6] E. M. GOLD, J. R. KENT and P. H. FORSHAM, Acta endocr., Copenh. *35*, 51 (1960).
[7] S. H. WAXMAN, D. F. TIPPIT and V. C. KELLEY, J. clin. Endocr. Metab. *21*, 943 (1961).
[8] N. M. KAPLAN, J. clin. Endocr. Metab. *23*, 945 (1963).
[9] J. J. CHART, H. SHEPPARD, M. ALLEN, W. L. BENCZE and R. GAUNT, Experientia *14*, 151 (1958).
[10] H. PARVEZ, S. PARVEZ and J. ROFFI, Archs int. Pharmac. Thér. *197*, 129 (1972).
[11] U. S. VON EULER and F. LISHAJKO, Acta physiol. scand. *45*, 122 (1959).
[12] M. W. MANGER, K. G. WAKIM and J. BOLLMAN, in *Chemical Quantitation of Epinephrine and Norepinephrine in Plasma* (Charles Thomas, Springfield, Illinois 1959).
[13] L. G. WHITBY, J. AXELROD and H. W. MALHERBE, J. Pharmac. exp. Ther. *132*, 193 (1961).
[14] G. COHEN and M. GOLDENBERG, J. Neurochem. *2*, 71 (1957).
[15] H. PARVEZ and S. PARVEZ, Hormone metab. Res., in press.
[16] J. J. PISANO, J. R. CROUT and D. ABRAHAM, Clin. chim. Acta *7*, 285 (1962).
[17] M. SANDLER and C. R. J. RUTHVEN, Biochem. J. *80*, 78 (1961).
[18] T. P. CONNELIAN and J. M. GODFREY, Clin. chim. Acta. *9*, 410 (1964).
[19] A. C. R. SLOSSE, C. r. Soc. Biol., Paris *97*, 1810 (1927).
[20] A. S. G. HUGGETT and D. A. NIXON, Lancet *11*, 368 (1957).
[21] R. J. WURTMAN and J. AXELROD, Biochem. Pharmac. *12*, 1439 (1963).
[22] H. PARVEZ and D. GRIPOIS, Clin. chim. Acta *17*, 39 (1972).

Table I.

Treatment group	Urinary excretion µg/kg/24 h				Blood/adrenaline (µg/l)				Glycemia (g/l)			Hepatic glycogen (g/kg liver)			
	Adrenaline	Noradrenaline	$\frac{A}{A+N}$	$\frac{A+N}{N}$	1 h	1.5 h	2 h	3 h	15 *M*	1 h	3 h	30 *M*	60 *M*	110 *M*	120 *M*
Controls	0.56±0.14 (35)	1.6 ±0.2 (35)	26% (35)	135% (35)	1.97±0.07 (12)				1.07±0.03 (15)			6.1±0.07 (9)			
Control+ metopirone	0.9 ±0.1 (36)	0.5 ±0.04 (36)	65% (36)	29% (36)	1.94±0.12 (8)	2.96±0.46 (8)	3.15±0.6 (8)	7.95±1.2 (8)	1.53±0.03 (5)	2.14±0.08 (7)	2.8±0.1 (6)	5.5 ±0.8 (5)	3.6±0.7 (5)	0.73±0.03 (5)	0.24±0.02 (5)
Adrenalectomized	0.05±0.02 (26)	2.12±0.03 (26)	22% (26)	102% (26)	1.65±0.1 (5)				1.01±0.05 (5)						
Adrenalectomized+ metopirone	0.18±0.02 (24)	1.94±0.08 (24)	8% (24)	109% (24)	1.6 ±0.08 (5)	1.68±0.06 (5)	1.62±0.09 (5)	1.7 ±0.1 (5)	1.13±0.12 (5)	0.9 ±0.07 (5)	0.91±0.09 (5)				

All values are highly significant from control groups ($P < 0.001$), A, Adr (Adrenaline), N, Noradr (Noradrenaline), ( ) No. of determinations.

Table II.

Monoamine oxidase in dpm in adrenals				Catechol-*o*-methyl transferase in dpm in adrenals				Vanylmandelic acid (µg in urine)			
Cont.	$^1/_2$ h	4 h	8 h	Cont.	$^1/_2$ h	4 h	8 h	Cont.	**A**	**H**	**H**+ACTH
2139 ± 43 (13)	2380 ± 97 (8)	3828 ± 15 (8)	2942 ± 17 (8)	967 ± 25 (8)	1025 ± 35 (8)	1257 ± 83 (8)	1936 ± 20 (8)	62 ± 3 (53)	96 ± 6 (28)	75 ± 3 (63)	54 ± 3 (18)

Cont. (normal rats), $^1/_2$, 4 and 8 h (h after 50 mg metopirone administration). **A**, adrenalectomized rats; **H**, hypophysectomized rats; **H**+ACTH, hypophysectomized rats injected with 4 U of ACTH. All the groups show highest degree of statistical significance.
MAO, $^1/_2$, 4 and 8 h differ from control ($P < 0.001$). COMT, $^1/_2$, 4 and 8 h also differs from control ($p < 0.001$). VMA, controls and adrenalectomized ($p < 0.001$); VMA, control and hypophysectomized rats.

Table II shows the activities of enzyme MAO and COMT in adrenal gland during 8 h of metopirone administration in dpm. At $^1/_2$, 4 and 8 h the activity of MAO rose to 111, 180 and 140% of its control values. The COMT activity also rose by 6, 30 and 100% during the above intervals. The urinary excretion of VMA/kg body wt./24 h in control, adrenalectomized and hypophysectomized rats injected with 4 IU of ACTH is also indicated in Table II. Hypophysectomy and adrenalectomy produced respective rises of 20 and 60% in VMA excretion. The treatment with ACTH only once declined the excretion of VMA to control level in hypophysectomized rats.

Discussion. Our results show that the decline in glucocorticoid concentration or their complete absence produces significant shifts in urinary and plasmatic catecholamines. These shifts in catecholamine excretion and release are immediately followed by their respective effects on hepatic glycogen and plasma glucose. Early studies[23] suggested that hormones of the adrenal cortex and catecholamines act as a single physiological unit. Many effects of catecholamines cannot be induced in the absence of corticosteroids[24]. HÖKFELT[25] reported that ACTH treatment influences the catecholamine levels in adrenals and heart. Studies performed during past few years provide sufficient evidence that the adrenal glucocorticoid hormones are of primary significance for the physiology of medullary chromaffin cells[26]. The decline in the concentration of glucocorticoids due to hypophysectomy severely effects adrenaline stores of adrenal gland[27,28]. The urinary excretion of adrenaline and noradrenaline in hypophysectomized humans during corticoid therapy provide supporting evidence that these hormones regulate excretion of catecholamines[29]. The studies on the role of glucocorticoids on adrenaline storage in adrenals or the maintenance of adrenal PNMT activity provide contrary evidence in catecholamine excretion, release and metabolism[2,27]. The inactivation of adrenal steroidogenesis by hypophysectomy declines the adrenal stores of adrenaline and PNMT activity significantly[2,4]. The administration of hydrocortisone or dexamethasone to hypophysectomized animals could maintain adrenal PNMT activity and adrenaline stores at control levels[2,4]. But the decline in glucocorticoids increases MAO and COMT activities. This suggests that these two enzymes, contrary to PNMT, have different hormonal specificities. PNMT is induced by glucocorticoids while MAO and COMT are inhibited by them. The higher urinary excretion of VMA in hypophysectomized and adrenalectomized rats provides good evidence for this observation. Recent studies[30,31] clearly indicate that adrenalectomy is followed by marked increase in cardiac MAO. Our experiments in progress show that adrenalectomy or hypophysectomy of the rat and rabbit foetuses or new-born results in significant rises in MAO and COMT activities in most of the body organs including the cerebral tissues.

Our preference for the use of Metopirone was based on the fact that it blocks the biosynthesis of corticoids immediately after its infusion and reduces cortisol to zero in just 4 h[8]. The rise in adrenaline urinary excretion after blocking glucocorticoid biosynthesis reflects that the presence of these hormones limits the excretion of adrenaline in blood which is the main source of urinary excretion of adrenaline[32]. The significant rise in urinary adrenaline of adrenalectomized rats after metopirone administration suggests that adrenaline from other sources of the body[33] was released by inhibition of extra-adrenal glucocorticoids. The rises in VMA excretion after hypophysectomy or adrenalectomy seem to be comparable with the concentrations of glucocorticoids in these two conditions. The corticoids are at the lowest level following adrenalectomy, while in hypophysectomized animals the adrenal cortex still functions and synthesizes these hormones at a lower intensity.

As the possible mechanisms by which glucocorticoids inhibit MAO and COMT activities, it could be suggested that the corticoids interfere with the synthesis of new enzyme protein. It appears that these hormones exert their effects at the level of RNA transcription from DNA[34].

These observations suggest that glucocorticoids which induce biosynthesis of adrenaline are equally important for the rate limiting control of catecholamine release, excretion and degradation.

Résumé. Nous avons étudié l'influence de l'inhibition de la biosynthèse des glucocorticoïdes par la Métopirone, sur la libération, la dégradation et l'excrétion des catécholamines chez le rat. Les résultats montrent qu'une diminution du taux de corticoïdes circulants chez des animaux normaux affecte profondément l'excrétion urinaire de l'adrénaline et de la noradrénaline. L'adrénalinémie est quadruplée quand le taux des corticoïdes est minimum. L'excrétion du VMA est augmentée de même que les activités monoamine-oxidase et catéchol-*o*-méthyl transférase.

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[23] E. R. RAMEY and M. S. GOLDSTEIN, Physiol. Rev. *37*, 155 (1957).
[24] S. W. BRITTON, Physiol. Rev. *10*, 617 (1930).
[25] B. HÖKFELT, Acta physiol. scand. suppl. *25*, 92 (1951).
[26] L. A. POHORECKY and R. J. WURTMAN, Pharmac. Rev. *23*, 1 (1971).
[27] R. J. WURTMAN, Endocrinology *79*, 608 (1966).
[28] J. ROFFI, J. Physiol., Paris *60*, 31 (1968).
[29] R. LUFT and U. S. VON EULER, J. clin. Endocr. Metab. *16*, 1017 (1956).
[30] V. M. AVAKIAN and B. A. CALLINGHAM, Br. J. Pharmac. exp. Ther. *33*, 211 (1968).
[31] T. C. WESTFALL and H. OSADA, J. Pharmac. exp. Ther. *167*, 2 (1969).
[32] P. HOLTZ, K. GREDNER and G. KRONEBERG, Arch. exp. Path. Pharmak. *204*, 228 (1947).
[33] J. AXELROD, Ciba Found. Study Group on Adrenergic Transmission *33*, 76 (1968).
[34] P. FEIGELSON and M. FEIGELSON, in *Action of Hormones on Molecular Processes* (Eds. G. LITWACK and D. KRITCHEVSKY; J. Willy and Sons, New York 1964), p. 218.

The Influence of Secretin on the Elimination of ^{14}C-Edrophonium in Bile

Although ^{14}C-edrophonium is mainly eliminated in bile as a glucuronide conjugate, small amounts of the unchanged drug can also be identified[1]. Biliary excretion of ^{14}C-edrophonium (but not of its metabolites) is influenced by the route of intravascular administration; after injection into the hepatic arterial tree, the proportion of the unchanged drug eliminated in bile is significantly greater than after i.v. injection[2]. The results of