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RADIATION-INDUCED RELEASE OF CATECHOLAMINES FROM PERFUSED BOVINE ADRENAL GLAND

by

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A decrease in the catecholamine content of the adrenal glands (GOODALL & LONG 1959) as well as increased urinary excretion of these amines (BRAUN & KUSCHKE 1961, FRANZEN et coll. 1963) may occur in several animal species after whole body roentgen irradiation. A post-irradiation rise in the urinary catecholamines has also been demonstrated in man (MCGOODALL 1968). Moreover, an enhanced liberation of catecholamines was observed when uterine horns of the rat were irradiated in vitro (VENINGA & BRINKMAN 1962).

With due regard to a previous finding of an increased release of catecholamines from isolated irradiated adrenal chromaffin granules (DEANOVIĆ & VENINGA) it seemed worthwhile to study the release pattern of catecholamines from the isolated perfused adrenal glands under the influence of roentgen irradiation.

BRINKMAN and his associates (BRINKMAN 1962, LAMBERTS & DIJKEN 1961, VENINGA 1965, BRINKMAN et coll. 1965) have maintained that the post-irradiation appearance and interaction of many free neurohormones and enzymes are involved in the primary irradiation effects. It was reasonable to expect in this experimental model an increased liberation of catecholamines after lower roentgen doses, as compared with those applied to the chromaffin granule suspensions.

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Table

Release rate of catecholamines from isolated perfused adrenal glands expressed in μg/min — Figures represent mean values ± standard errors

	(1) —10 to 0 min before	(2) 0 to 10 min after	(3) 10 to 20 min after	Differences (2)—(1)	(3)—(1)	(3)—(2)
Irradiation						
1 000 R (N=6)	7.09±2.18	8.10±2.55	8.69±2.40	1.05±0.52 ↑ n.s.	1.61±0.45 ↑ P<0.02	0.59±0.71 ↑ n.s.
Sham-irradiation				↓	↓	↓
Control (N=4)	12.76±1.65	12.51±2.35	11.38±2.54	—0.25±0.86	—1.13±0.1	—0.64±0.52

Materials and Methods. The left, kidney-shaped, bovine adrenal glands were removed and cooled in ice within 30 minutes of slaughtering the animals; quick transport to the laboratory followed.

The method of retrograde perfusion through the central adrenal vein, as originally described by HECHTER et coll. (1953) and adapted by DOUGLAS & RUBIN (1961), HAAG et coll. (1961), and SCHÜMANN & PHILIPPU (1963), was applied. This simple method consists of rapid removal of the periadrenal fat and connective tissue, followed by cannulation of the large central vein. A 2 to 3 mm deep incision at the opposite side of the gland insures that the perfusate will escape. The gland was mounted in a plastic organ chamber and perfused with Tyrode solution of 39°C and previously saturated with carbogen gas; the perfusion pressure was 55 to 60 cm water. The gland was warmed up and the remaining blood rinsed out during the first three minutes of perfusion. Clear drops of Tyrode solution formed at the incision were collected in a measuring cylinder containing 0.35 ml of concentrated $HClO_4$. The perfusion rate was adjusted to approximately 10 ml/5 min by altering the pressure.

Two 10 ml samples were collected and the gland was irradiated with a Siemens Dermopan roentgen generator. Irradiation conditions were 50 kV, 25 mA, 1 mm Al filter, tube diameter 4 cm, focus-to-gland distance 2.5 cm, and dose rate 870 R/min, as measured in the water-filled organ chamber. The perfusion was not interrupted during the irradiation.

Four samples of 10 ml perfusate were collected in the course of 20 ± 2 minutes after irradiation.

The pH of the samples was adjusted to 6.0 by means of a 20 % K_2CO_3 solution; the precipitate was removed by subsequent centrifugation. The procedure of ATKINSON & WYNNE (1962) was applied for the absorption and elution

of the catecholamines. The fluorimetric method of SHORE & OLIN (1958) was employed to determine both the adrenalin and noradrenalin together with a Locarte semi-spectrofluorimeter. (LF-1 and B390 filters on the primary side produced an excitation at 400 mμ; the monochromator on the secondary side was adjusted to 520 mμ.) The standard solution contained 2 μg/ml adrenalin, the blank consisting of 0.4 N sulfuric acid. The relationship between the adrenalin concentrations below 2 μg/ml and the fluorimeter readings was linear.

Results and Discussion

A dose of 1 000 R was found to be the lowest one giving a measurable effect after a number of pilot experiments.

The concentration of catecholamines determined in the perfusate samples before irradiation varied in the experimental group between 0.2 and 1.4 μg/ml with an average value of 0.6 μg/ml; post-irradiation values ranged from 0.3 to 2.1 μg/ml giving a mean of 0.9 μg/ml. In the sham-irradiated group the concentration of these amines before 'treatment' varied between 0.5 and 1.7 μg/ml with a mean of 1.1 μg/ml, and thereafter between 0.3 and 1.7 μg/ml giving a mean of 1.0 μg/ml.

The data obtained were recalculated in μg/min to correct the small differences in collection time. The average values of the rate of release obtained in every 10-minute period are presented in the Table. It is seen that the yield of catecholamines in irradiated glands progressively increased towards the end of the 30-minute observation period. In contrast, the leakage of catecholamines from the sham-irradiated glands steadily decreased. Only the differences in the rate of catecholamine release between the third and the first collection periods were significantly different in the irradiated and sham-irradiated adrenals ($P < 0.02$, student's t-test). A distinct variation in the starting values of the release rate of catecholamines was observed between the groups.

A dose as high as 1 000 R to the isolated perfused adrenal gland provokes a significant increase in catecholamine liberation. As compared with our previous results with isolated chromaffin granules (DEANOVIĆ & VENINGA) this dose is 3 to 4 times lower. However, in the case of whole body exposure a dose of only 400 R leads to a significant rise in the content of urine catecholamines (FRANZEN et coll. 1963; GOODALL 1968). This suggests the existence of a gradually increasing radiation responsiveness when the release takes place in isolated subcellular elements, complete organs, or in the integral organism. This concept of enhanced radiation response is consistent with the idea of the potentiating interaction of liberated neurohormones and enzymes (BRINKMAN 1962, BRINKMAN et coll. 1965). Taking into consideration the possibility of a simultaneously radi-

ation-induced release of several neurohormones (LAMBERTS & DIJKEN 1961, VENINGA 1965), it seemed reasonable to expect a liberation of acetylcholine from splanchnic nerve intramedullary terminals in the experimental object as well as in the whole organism. The secretion mechanism of catecholamines (DOUGLAS 1966, SMITH 1968) might consequently be set in motion.

The initial release of catecholamines in both groups of glands was distinctly different, to which three factors in particular may have contributed: (1) experiments with irradiated glands and control experiments were perforce not run at the same time, (2) the duration of the terminal asphyxia in the slaughtered cattle could not be controlled so that differences in the discharge of catecholamines by agonal sympathetic nerve stimulation might have occurred, (3) the time interval between slaughtering and the start of perfusion varied from 1 to 2 hours, and the duration of the gland cooling corresponded with this time interval.

It is well known that the adrenal medulla is not essential to life if the organism is unexposed to stress. Since adrenalectomized animals exhibit increased radiosensitivity (CRONKITE & CHAPMAN 1950, EDELMAN 1951, BETZ 1956), there is justification for considering irradiation as a stressful condition (GOODALL & LONG 1959, BACQ & ALEXANDER 1961, BRINKMAN 1962) characterized by a typical quick neuro-endocrine reaction. Although the 'classical' concept of an adrenalin-hypothalamus-pituitary-adrenal cortex chain mechanism has been laid open to criticism (VOGT 1952, GUILLEMIN 1955), it seems at present very likely that free adrenalin exerts a direct influence on the reticular activating system (ROTHBALLER 1959). With due regard to the hypothesis that the autonomic nervous system of vertebrates is more easily influenced by irradiation than the central nervous system (HUG 1962), the following concept might be deduced from the results.

Catecholamines are liberated by a direct radiation effect on the adrenal medulla; the free adrenalin then sets in motion the complex of neuro-endocrine reactions via the reticular formation. In addition, if whole body irradiation is considered a 'distributed stimulus' (HUNT & KIMELDORF 1964), it is reasonable to suppose a simultaneous central (hypothalamic) as well as a peripheral (adrenal + sympathetic) release of neurohormones leading to mutually potentiating interactions. These events might be interpreted as the principal pathogenetic factors in early radiation sickness (BRINKMAN 1962). Evidence exists that these early reactions to irradiation could exert a favourable influence with regard to the survival of the organism (BACQ et coll. 1960).

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SUMMARY

Isolated bovine adrenal glands were perfused through the central adrenal vein and irradiated with 1 000 R roentgen. The catecholamine content in aliquots of the perfusate after irradiation presented a gradually increasing trend, in contrast to the decrease observed in sham-irradiated controls. A significant difference in the release rate of catecholamines existed between the two groups. The findings are discussed in relation to the possible role of catecholamines in the early neuro-endocrine reactions to irradiation.

ZUSAMMENFASSUNG

Isolierte Rindernebenniere wurden durch die zentrale Nebennierenvene retrograd durchströmt und mit 1 000 Röntgen bestrahlt. Der Brenzcatechinamingehalt in Aliquoten der Durchströmungsflüssigkeit nach der Bestrahlung zeigte eine allmählich zunehmende Tendenz im Gegensatz zu einer abnehmenden Tendenz bei den nicht-bestrahlen Kontrollen. Ein signifikanter Unterschied in der Freisetzungsgeschwindigkeit der Brenzcatechinamine wurde zwischen den beiden Gruppen festgestellt. Die Befunde werden mit Hinsicht auf die mögliche Rolle der Brenzcatechinamine in der neuro-endokrine Bestrahlungsfrühreaktion diskutiert.

RÉSUMÉ

Les auteurs ont perfusé par la veine surrénale centrale des glandes surrénales isolées de bœuf et les ont irradiées par 1 000 roentgen. La concentration en catécholamines du liquide de perfusion après irradiation tend à augmenter graduellement, contrairement à la diminution observée sur les surrénales témoins soumises à une irradiation simulée. Il y a une différence significative du taux d'excrétion de catécholamines entre les deux groupes. Les auteurs examinent ces résultats et les rapprochent du rôle possible des catécholamines dans les réactions neuro-endocriniennes précoces à l'irradiation.

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