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EXCRETION OF METABOLITES OF BIOGENIC AMINES IN PATIENTS WITH IRRADIATED BRAIN TUMOURS

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An increased excretion of biogenic amines has been found in different mammals after irradiation. Thus the urinary catecholamines are increased in the irradiated rat (BRAUN & KUSCHKE 1961, FRANZEN et coll. 1963) as well as in man (MCGOODALL 1968). The excretion of 5-hydroxy-tryptamine was also shown to be enhanced (FRANZEN et coll.). The results of other authors indicate that the irradiation provokes the release of the biogenic amines from their storage sites (MCGOODALL & LONG 1959, WILLOUGHBY 1960, PALAIĆ & SUPEK 1965, VARAGIĆ et coll. 1967), after which they appear in the blood and urine. These monoamines are being to a great deal metabolized, so that their final metabolites are excreted in the highest amount (in free as well as in the conjugated form). Some of these metabolites, for instance 5-hydroxy-indoleacetic acid (5-HIAA), are increased in the whole-body irradiated rabbit and rat (RENSON & FISCHER 1959, MELCHING et coll. 1960, RANDIĆ & SUPEK 1961). Moreover, this metabolite is excreted in enhanced amount in most patients submitted to the therapeutical partial-body irradiation (SMITH & LANGLANDS 1966, PERIČIĆ & DEANOVIĆ 1973). Patients with therapeutically irradiated abdomen have a more intensive excretion of catecholamine metabolites (PERIČIĆ 1972). Since biogenic

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amines play an important neurotransmitter role in the central nervous system, the investigation of the excretion of their metabolites in the patients with irradiated brain tumours seemed to be interesting. Data about the radiation sensitivity of the brain tissue are rather controversial. Light microscopy has indicated that very high doses of ionizing radiation are required to impair the structure of the nervous system. Therefore, the nervous tissue was considered to be very resistant to radiation. Later it was found that the brain functions and behaviour may be altered with very small doses of radiation, and the previous conception was submitted to criticism (LEBEDINSKY et coll. 1958, GANGLOFF & HALEY 1960, HUNT & KIMELDORF 1964). Blood vessels and glial cells were found to be more susceptible to the radiation injury than neurons, and white matter more than gray (RUBIN & CASARETT 1968). If the neurons are directly injured by irradiation or if the nerve cell injury is secondary to the impairment of blood vessels or glial cells, is not definitively known as yet. Anyway, the described abnormalities provoked by irradiation have in its biochemical basis the release of neurotransmitter substances such as biogenic amines.

Material and Methods

Thirteen patients of different sex and age and with brain tumours of various location and type were irradiated (Table 1). Before irradiation the tumour tissue was removed as radically as possible in all cases. Fifteen to 30 days postoperatively, the operated region was submitted to telecobalt therapy (Gammatron 3, Siemens). The irradiated field was always 3 cm wider than the border of the removed tumour tissue. The remaining part of the head was shielded. The dose was dependent upon the clinical condition of the patient; those in relatively good general condition, and without signs of disturbed brain function, were given higher daily doses from the beginning; those with symptoms or signs of some brain disturbance (related to increased intracranial pressure or similar), were irradiated with more caution because of the possibility of inducing brain oedema. Thus, each patient had his own schedule of irradiation. The dose-rate was 100 R/min, and focus to skin distance 75 cm.

24-hour urine of these patients was collected under toluene in the control period (3 days before irradiation) as well as during the first 3 days of irradiation. This whole time the patients were on a diet avoiding food and drinks which might have some influence on the metabolism of the determined compounds.

The following metabolites of biogenic amines were determined in urine: 5-hydroxyindoleacetic acid (5-HIAA)—the main metabolite of 5-hydroxytryptamine (DALGLIESH 1958), the metabolites of catecholamines i.e. vanillinmandelic acid (VMA) and free 3-methoxy-4-hydroxy-phenylglycol (MHPG) both by the method of SAPIRA (1968). In all urine samples creatinine was also determined as the referent substance (FOLIN 1954).

The results are evaluated statistically by the t-test using 'method of differences' which is specially convenient for small sample of dependent variates (FISCHER 1950).

Table 1

The material of patients with irradiated brain tumours

Case No.	Age and sex	Brain tumour (Type and location)	Irradiation		
			Area	Field size (cm)	Dose (R)
1	63 M	Gliosarcoma (temp. occipit.)	Temporooccipital	10 × 9	200, 200, 200
2	31 M	Oligodendroglioma malignum (front. pariet.)	Frontoparietal	8 × 10	100, 100, 100
3	9 F	Medulloblastoma (vermis)	Posterior cranial fossa	11 × 7	300, 300, 300
4	54 F	Glioblastoma (front. temp.)	Frontotemporal	8 × 10	100, 200, 300
5	26 M	Reticulosarcoma (front.)	Frontotemporal	9 × 9	100, 100, 150
6	29 M	Transitional oligodendroglioma	Parietotemporo-occipital	10 × 13	50, 100, 100
7	30 M	Recurrent transitional oligodendroglioma (pariet. temp.)	Parietotemporal	11 × 11	50, 100, 100
8	48 M	Malignant tumour (meningiosarcoma?)	Frontotemporal	8 × 11	200, 200, 200
9	16 F	Cerebellar sarcoma	Posterior cranial fossa	8 × 10	300, 300, 300
10	67 F	Pituitary adenoma	Temporal	5 × 5	200, 200, 200
11	34 F	Gliosarcoma (pariet. temp. occipit.)	Parietotemporo-occipital	10 × 14	400, 400, 500
12	60 F	Glioblastoma (pariet. temp. occipit.)	Parietotemporo-occipital	16 × 12	500, 500, 500
13	43 F	Mixed transitional glioma (pariet. occipit.)	Parietotemporo-occipital	10 × 13	400, 400, 400

Results

The excretion of the determined metabolites of biogenic amines in the patients with irradiated brain tumours appears in Table 2. The control values represent the mean 24-hour excretion of the related metabolites in all patients during the 3 pre-irradiation days. The quantities of the excreted metabolites in the course of radiation therapy are presented for all patients together as the mean excretion for each of the 3 first days separately. The highest 5-HIAA excretion as well as that of MHPG occurred on the second day of irradiation, but the VMA excretion increased gradually until the third day. In some of these patients the excretion of the mentioned metabolites was followed for longer than 3 days, but these values are again in the range of the control ones. Fig. 1 demonstrates the excretion of 5-HIAA in each of 12 patients with irradiated brain tumours. If two groups of 5-HIAA values (control and irradiated) are compared, a significant 5-HIAA increase ($p < 0.02$) during irradiation is found. When the 5-HIAA excretion of each day of irradiation is compared separately

Table 2

The metabolites of biogenic amines in urine (Mean values ± S.E.)

	5-HIAA μg/mg creatinine (N = 12)	VMA μg/mg creatinine (N = 13)	MHPG μg/mg creatinine (N = 13)
Control*	3.321 ± 0.314	3.478 ± 0.244	0.863 ± 0.055
Radiation therapy			
First day	3.906 ± 0.682	4.054 ± 0.419	0.940 ± 0.136
Second day	4.623 ± 0.606	4.618 ± 0.762	1.287 ± 0.180
Third day	3.864 ± 0.681	5.018 ± 0.883	1.135 ± 0.213

* Mean value of 3 pre-irradiation days ± S.E.

with control values, a more significant 5-HIAA increase ($p < 0.01$) appears on the second day of treatment (Fig. 2).

The comparison of MHPG excretion in the pre-irradiation period and MHPG excretion on the second day of irradiation (Fig. 3) also shows a significant difference ($p < 0.05$). However, the mean excretion value of this metabolite during three days of irradiation does not show any significant difference compared to the control value.

The excretion of another catecholamine metabolite—VMA—in the same patients is presented in Fig. 4. The mean VMA values obtained during the 3 days give a significant difference ($p < 0.05$) in comparison to the control values. The highest mean VMA excretion (Table 2) is expressed on the third day, but because of the great variations this difference is regarded as non-significant.

Discussion

It is well known that whole-body irradiation of animals is followed by a decrease of 5-hydroxytryptamine in brain tissue (ERSCHOFF et coll. 1962, PALAIĆ & SUPEK, PAUSESCU et coll. 1973). A diminished 5-hydroxytryptamine concentration after the irradiation occurs also in other organs e.g. spleen and small intestine, and this leads to an increase of this metabolite in urine. Enhanced 5-HIAA excretion has been observed in most patients locally irradiated (SMITH & LANGLANDS, PERIČIĆ & DEANOVIĆ). In the present patients, the increased amount of 5-HIAA in urine is presumably due to a local release of 5-hydroxytryptamine from the irradiated brain tissue. The excretion of 5-HIAA is increased specially on the second day of radiotherapy ($p < 0.01$). This finding is in accordance with the data of other authors (WILLOUGHBY, PALAIĆ & SUPEK), who have shown the maximum drop of 5-hydroxytryptamine 48 hours after irradiation in the intestine as well as in the brain. Moreover, in the present patients an additive effect of 2 initial radiation doses might occur.

It seems that the significantly increased excretion of noradrenaline metabolite

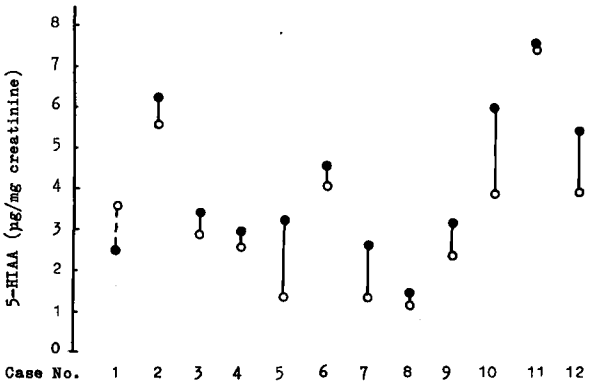

Fig. 1. 5-HIAA excretion. Mean values of 3 days obtained in the course of irradiation (●) are compared to mean values of 3 days in the control period (○). ○—● positive difference; ●---○ negative difference ($p < 0.02$).

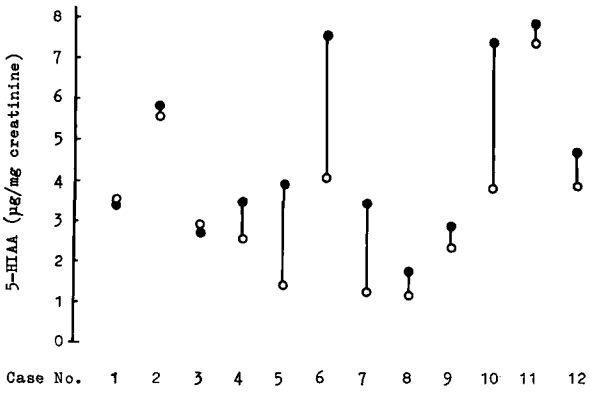

Fig. 2. 5-HIAA excretion. The second day of irradiation (●) is compared to mean values of 3 days in the control period (○). ○—● positive difference ($p < 0.01$).

MHPG in the second day of irradiation might be explained in a similar way. It is known that the quantity of catecholamines is decreased in the organs of irradiated animals (McGoodall & Long, Varagić et coll., van Woert & Korb 1970) and increased in the urine (Braun & Kuschke, Franzen et coll., McGoodall). As a reflection of the local release of catecholamines in patients with irradiated abdomen (Ra-therapy) a significantly enhanced excretion of all catecholamine metabolites, except MHPG, has been found (Perič̆ić). This might indicate a different origin of MHPG from that of other catecholamine metabolites. The hypothesis about the most central origin of MHPG was supported by many authors (Mannarino et coll. 1963, Maas & Landis 1966, 1968, Schanberg et coll. 1968, Scharman 1969, Maas et coll. 1972). Significant increase of this metabolite in the patients with irradiated brain tumours would also lend support to this hypothesis. Certainly the mentioned increase could be more expressed in the case that the estimation of the total (i.e. free plus conjugated) urinary MHPG has been possible.

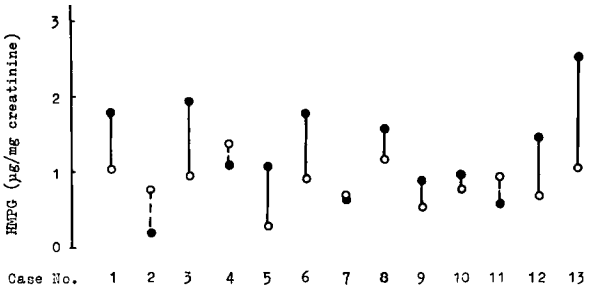

Fig. 3. MHPG in urine. The second day of irradiation (●) is compared to mean values of 3 days in the control period (○). ○—● positive difference; ●---○ negative difference ($p < 0.05$).

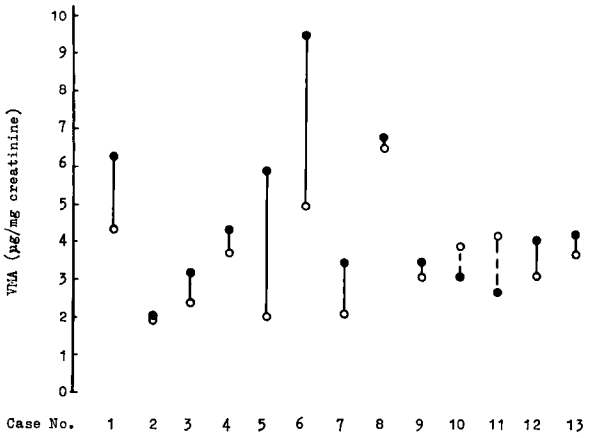

Fig. 4. VMA in urine. Mean values of 3 days obtained in the course of radiotherapy (●) are compared to mean values of three days in the control period (○). ○—● positive difference; ●---○ negative difference ($p < 0.05$).

However, the excretion of VMA during the course of irradiation is different. The excretion of this catecholamine metabolite is significantly increased during the whole period (3 days of irradiation), whereas the VMA excretion in none of the single days has been markedly aberrant from the control, but it climbs gradually till the third day of irradiation. The reason for the delayed and progressively intensified VMA excretion may be due to the secondary inclusion of the peripheral sympathetic system in the response of the organism to the irradiation of brain tumours. It has been shown that the sympathetic adrenergic nerves contribute only to a very small extent to the total number of brain noradrenaline nerve terminals (SACHS & JONSSON 1973). A greater part of adrenergic nerves in the brain represent the ascending axons belonging to the dorsal noradrenaline bundle (UNGERSTEDT 1971). It is possible that the estimated MHPG reflects the released noradrenaline from both groups of adrenergic terminals in the brain. On the other hand, the enhancement of the VMA excretion, mainly the metabolite of the peripheral released noradrenaline (MAAS et coll.), may not occur before the activation of the peripheral sympathetic adrenergic system takes place. For a general reaction of the whole sympathetic adrenergic system (central and

peripheral) pleads also the uniform reaction of this system in response to stimulation by drugs (VOGT 1954), as well as in response to stress induced by different physical stimuli (MAYNERT & LEVI 1964). The stimulation of the peripheral sympathetic neurons during the course of irradiation of brain tumours may occur in a neural way; it is known that an intimate synaptic contact between the endings of noradrenaline containing neurons and the sympathetic preganglionic neurons exists in the intermediolateral columns of the spinal cord (DAHLSTRÖM & FUXE 1965).

Although the clinical material was fairly heterogenous, some comparisons between the similar cases and their biochemical reactions are possible. However, any explicit dependency of the monoamine metabolites excretion on the type and location of tumour was not observed. No correlation existed neither in comparison with the irradiated surface and the dose, nor in comparison with the radicalness of the operation. In some patients a marked clinical improvement during the first days of irradiation was accompanied by an excessive increase in the excretion of all metabolites examined (for instance in case 5), but this relationship was not clear in other cases. Attempts to correlate the biochemical response with the appearance or absence of early signs of radiation sickness (nausea, vomiting, etc.) did not give positive results. The excretion of metabolites of biogenic amines in the patients with irradiated brain tumours was neither dependent on the time interval between the operation and the beginning of radiation therapy. The sham irradiation, which has been done in some cases, did not change the excretion of metabolites of biogenic amines; thus the above described phenomena are certainly not a nonspecific stress reaction but a process directly induced by irradiation and submitted to individual variations.

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SUMMARY

The metabolites of biogenic amines were determined in the 24-hour urine samples of patients submitted to surgical removal of a malignant brain tumour and subsequently to telecobalt therapy of the corresponding head region. A significant increase in the excretion of 5-hydroxyindoleacetic acid (5-HIAA), vanillinmandelic acid (VMA) as well as of free 3-methoxy-4-hydroxy-phenylglycol (MHPG) during the period of irradiation was found. This increase is presumably the result of radiation induced release of their parent amines from the brain; in the case of VMA the secondary response of the peripheral sympathetic system might occur.

ZUSAMMENFASSUNG

Es wurden die Metaboliten biogener Amine in 24-Stunden Urin Proben von Patienten, die nach chirurgischer Entfernung eines malignen Gehirntumors nachfolgend einer Telecobolt Therapie der entsprechenden Kopf Region ausgesetzt worden waren, untersucht. Es wurde ein signifikanter Anstieg der Ausscheidung von 5-Hydroxyindolessigsäure (5-HIAA), Vanilinmandelsäure (VMA) sowie von freiem 3-Methoxy-4-Hydroxy-Phenolglykol (MHGG) während der Bestrahlungs Periode gefunden. Dieser Anstieg ist häuptsächlich das Ergebnis einer Strahlen-bedingten Freisetzung der ursprünglichen Amine vom Gehirn; im Fall von VMA mag eine sekundäre Antwort des peripheren sympathischen Systems vorliegen.

RÉSUMÉ

Les auteurs ont dosé les métabolites des amines biogéniques sur des prélèvements d'urine de 24 heures de malades soumis à une exérèse chirurgicale d'une tumeur cérébrale maligne, puis traités par télécobalthérapie de la région correspondante de la tête. Ils ont constaté une augmentation importante de l'excrétion de l'acide 5-hydroxyindoleacétique (5-HIAA), de l'acide vanillinemandélique (VMA) ainsi que du 3-méthoxy-4-hydroxy-phénylglycol libre (MHPG) au cours de la période d'irradiation. Cette augmentation est vraisemblablement le résultat d'une libération, sous l'effet des radiations, à partir du cerveau, de leurs amines parentes; dans le cas du VMA, pourrait intervenir la réponse secondaire du système sympathique périphérique.

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