Biochemical Pharmacology, Vol. 33, No. 9, pp. 1411–1415, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00

EXCRETION OF CATECHOLAMINES AND THEIR METABOLITES IN TRANSPLANTABLE RAT PHAEOCHROMOCYTOMA

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Abstract—The urinary excretion pattern of catecholamines and their metabolites was studied in rats bearing a subcutaneous transplantable phaeochromocytoma. Compared with normal rats, tumour-bearing animals showed a markedly raised excretion of dopamine, noradrenaline and adrenaline, together with certain of their major acidic and alcoholic metabolites. No evidence of increased octopamine production could be obtained. There was a significant correlation between the output of dopamine and its metabolites, allowing accurate assessment of dopamine turnover rates which were comparable with those observed in human phaeochromocytoma. Tumour development, as determined by tumour weight, also correlated significantly with urinary excretion of noradrenaline and dopamine. Rat phaeochromocytoma appears to be a useful model for the human tumour.

An animal model of phaeochromocytoma first became feasible when Gillman *et al.* [1] noted the spontaneous occurrence of these tumours in Wistar rats. It became available as an experimental tool after a high incidence of tumour formation had been observed in elderly irradiated NEDH rats [2]. A more reproducible model system is based on tumour cells arising from irradiated animals [3, 4] or from cultured rat PC12 tumour cells [5, 6] which can be transplanted into healthy rats. Such cells have previously been reported predominantly to produce dopamine and its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and 3,4-dihydroxyphenylethanol (DHPE) as well as noradrenaline and 3,4-dihydroxyphenylglycol (DHPG) [7].

Tumour-bearing rats show many characteristics of the human disease, including elevated blood pressure [3, 8], raised urinary 4-hydroxy-3-methoxymandelic acid (VMA) and metadrenalines [3], raised plasma and urinary catecholamines [6, 9], weight loss, proteinuria and renal and cardiac lesions [3]. In view of the relative paucity of data concerning the urinary output of catecholamines and metabolites in normal and tumour-bearing rats, we have further characterized the excretory pattern in these animals.

MATERIALS AND METHODS

The tumour used in these experiments was that described by Warren and Chute [3] derived from X-irradiated NEDH rats [2] and characterized histologically by De Lellis *et al.* [10]. The excised tumour was coarsely minced and suspended in physiological normal saline before being transplanted by subcutaneous injection into healthy male rats of the same strain [3]. After 4–5 weeks, 24 hr urinary output from 15 tumour-bearing and 14 control animals was collected quantitatively in metabolic cages. Acidified samples were stored at −20° until assay. The transplanted tumours were excised, coarsely dissected free from normal adjacent tissue, weighed, minced and frozen at −20° prior to analysis.

Free catecholamines were assayed in urine and tissue extracts by a modification of the radioenzymatic method of Callingham and Barrand [11] and Martin *et al.* [12]. Approximately 0.5 g wet tumour tissue was homogenized in 1 ml of 0.2 M perchloric acid containing 0.1% w/v EDTA and 1 mM ascorbic acid and centrifuged at 30,000 *g* for 30 min. Following protein determination, the supernatant was diluted with 0.2 M perchloric acid to a concentration of 6 μg protein in 10 μl perchloric acid. A 10 μl aliquot of tissue supernatant or 10 μl acidified urine sample containing 1 mM ascorbic acid and 0.1% w/v EDTA was diluted with water to 180 μl and incubated for 60 min at 37° with 130 μl of a solution containing 25 mM $MgCl_2$, 0.1 mM pargyline, 0.5 mM dithiothreitol, 0.9 μM (^{3}H)*S*-adenosylmethionine, 0.7 mg partially purified rat liver catechol *O*-methyltransferase preparation [13], 195 mM Tris-base (pH 9.3) and 2 mM ethylene glycol-bis-*N*,*N*′-tetraacetic acid, and then worked up according to Martin *et al.* [12]. Substrate-depleted blanks consisting of diluted tissue supernatants or urine containing 1 mM ascorbic acid and 0.1% w/v EDTA were passed through an alumina column following adjustment to pH 8.6 to remove catechols prior to incubation.

Tumour noradrenaline and adrenaline were assayed by the fluorimetric method of Anton and Sayre [14] as a comparison. Good agreement was obtained between the two techniques.

Phenolic acids and alcohols were measured in urine after hydrolysis overnight with a sulphatase–

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glucuronidase preparation (suc *d'Helix pomatia*, Industrie Biologique Française, Villeneuve la Garenne, France) at pH 6.0 and 37°. Phenolic acids were extracted into ethyl acetate from the hydrolysed urine samples after acidification. They were esterified with ethanolic HCl and purified on an anion exchange resin (AG 1-X4). These were silylated and then estimated by gas chromatography using a capillary column and flame ionization detection [15]. Phenolic alcohols were extracted into ethyl acetate from the hydrolysed urine at pH 7.5, isolated on AG 1-X4 resin [16] and estimated gas chromatographically as their silyl ethers using a capillary column and flame ionization detection [15]. Protein was determined by the method of Lowry *et al.* [17], using bovine serum albumin as standard.

RESULTS

Tumour-bearing animals showed a 5- to 60-fold increase in mean excretion of all compounds measured except *p*-hydroxymandelic acid (p-HMA) (Table 1). The effect was most marked for noradrenaline and 4-hydroxy-3-methoxyphenylethanol (HMPE) which showed 58- and 30-fold increases respectively, with about a 5-fold increase for adrenaline and DOPAC.

DHPG was not detected in urine from control animals, but output reached a mean of 43.4 μg/24 hr in the five tumour-bearing rats tested. VMA and 3,4-dihydroxymandelic acid (DOMA) could not be detected in either group of animals. VMA excretion in normal rats is very low [18] and technical problems precluded the measurement of the low concentrations of VMA in experimental or control animals by GC. The identity of each alcoholic metabolite was confirmed by gas chromatography–mass spectrometry.

In general, metabolites were excreted in greater quantities than their parent amines, both in control and experimental animals (Table 1). In 80% of the tumour-bearing animals the output of noradrenaline was as much as four times that of dopamine. Adrenaline output was always lower than that of the other catecholamines. The urinary output of 4-hydroxy-3-methoxyphenylglycol (HMPG) in tumour-bearing animals was considerably greater than that of VMA (100 μg/24 hr excretion would have been measurable), confirming that the glycol is the major breakdown product of noradrenaline in rat urine, in contrast to dopamine where the range in ratios of HVA plus DOPAC to HMPE was 9.7–60. Thus, tumour-bearing animals as well as control animals excreted relatively more alcoholic than acidic metabolites of noradrenaline, whereas the reverse is true for dopamine.

Correlations between various parameters in control and experimental animals were sought (Table 2). Because of the correlation between urinary dopamine and its metabolites, we were able to calculate daily turnover rates for dopamine [19]. The rates were 1000%, 780% and 830% respectively for animals No. 10, 13, and 14 (Table 3).

DISCUSSION

Earlier observations on the excretion pattern of catecholamines [9] and their metabolites, VMA and the metadrenalines [3] in rat phaeochromocytomas were fragmentary. We have been able to confirm the raised catecholamine but not VMA excretion. The increase in adrenaline excretion observed here, albeit relatively modest, is consistent with the measured increase in 42% of phaeochromocytoma patients [20]. However, unlike the clinical situation, where only about 10% of cases have dopamine-secreting tumours, all tumour-bearing rats showed a substantial rise in urinary dopamine output, which correlated with noradrenaline excretion. Nonetheless, noradrenaline excretion is still four times greater than dopamine excretion in tumour-bearing rats, a pattern which is also reflected in the relative output of their respective metabolites HMPG and HMPE. However, in control rats, dopa-

Table 1. Urinary monoamines and their metabolites in phaeochromocytoma-bearing rats and normal controls

Metabolite	Control rats (N = 14) (μg/24 hr) Mean ± S.E.M.	Range	Tumour-bearing rats (N = 15) (μg/24 hr) Mean ± S.E.M.	Range
Dopamine	2.18 ± 0.12	1.35–3.00	48.7 ± 17.1	6.0–281
Noradrenaline	1.15 ± 0.067	0.79–1.63	58.6 ± 7.95	17.0–155
Adrenaline	0.53 ± 0.068	0.21–1.04	2.49 ± 0.33	1.31–6.66
4-Hydroxy-3-methoxy-phenylglycol	25.6 ± 3.54	7.0–59	409 ± 54.6	108–760
4-Hydroxy-3-methoxy-phenylethanol	2.3 ± 0.69	<1–11	71.7 ± 15.1	13–230
Homovanillic acid	30.8 ± 4.81	10–87	536 ± 130.6	82–1820
3,4-Dihydroxyphenyl-acetic acid	25.7 ± 2.45	17–49	181 ± 35.9	51–500
p-Hydroxymandelic acid	51.4 ± 3.55	24–70	56 ± 5.19	17–94

Table 2. Correlations between measured parameters

Animal	Parameter A	Parameter B	r	P
Control	Noradrenaline	HMPG	0.57	<0.05
Experimental	Noradrenaline	HMPG	0.38	>0.05
Control	Dopamine	Total dopamine metabolites	0.31	>0.05
Experimental	Dopamine	Total dopamine metabolites	0.74	<0.001
Control	HMPG	HMPE	0.26	>0.05
Experimental	HMPG	HMPE	0.35	>0.05
Control	Dopamine	Noradrenaline	0.40	>0.05
Experimental	Dopamine	Noradrenaline	0.88	<0.001
Control	HVA + DOPAC	HMPE	0.79	<0.001
Experimental	HVA + DOPAC	HMPE	0.89	<0.001
Experimental	Noradrenaline*	HMPG	0.24	>0.05
Experimental	Noradrenaline*	Noradrenaline	0.57	>0.05
Experimental	Tumour wt	Noradrenaline	0.89	<0.001
Experimental	Tumour wt	Dopamine	0.96	<0.001
Experimental	Tumour wt	HMPE	0.83	<0.001
Experimental	Tumour wt	HVA + DOPAC	0.58	<0.05
Experimental	Tumour wt	HMPG	0.35	>0.05
Experimental	Tumour wt	Noradrenaline*	0.10	>0.05

* Amount in tumour. All other metabolite measurements are in urine.

mine and HMPE excretion exceed that of noradrenaline and HMPG respectively. Endogenous synthesis of dopamine from L-dopa in the kidney may contribute to its excretion. The predominance of noradrenaline relative to dopamine excretion in tumour-bearing rats is consistent with similar ratios in a clonal cell line established from this tumour [21]. On the other hand, rat tumour suspensions contain relatively more DOPAC than DHPG, the major metabolites of dopamine and adrenaline, respectively, in these cells [7]. This anomalous observation may be due to the preferential metabolism of dopamine by MAO in these cells [7].

Despite relatively high dopamine output, there was no correspondingly high concentration of dopamine in tumour tissue to match the elevated noradrenaline level, suggesting that dopamine storage ability is limited. The high dopamine turnover rates observed here are also consistent with this. In one particular clonal cell line [5] where dopamine levels are relatively high compared with noradrenaline, both amines are released in equal amounts [22]. Thus the relative amounts of these amines, secreted both *in vitro* and *in vivo*, appear to bear no simple relationship to their intracellular ratio. Urinary excretion of catecholamines is also independent of tissue concentration since we found no correlation between tumour noradrenaline level and either its urinary excretion or that of its alcoholic metabolite, HMPG. Nor was there any correlation between tissue noradrenaline concentration and tumour weight.

These correlations have not previously been evaluated in the rat, although in human phaeochromocytoma Crout and Sjoerdsma [19] noted a correlation between tumour weight and catecholamine concentration; large tumours, they claimed, are associated with higher catecholamine concentrations and a greater proportion of metabolites, because of a low rate of turnover of catecholamine stores. These findings have subsequently been questioned, since no correlation was found between tumour weight and tumour catecholamine storage ability [23–25] or urinary catecholamine and metabolite excretion [24, 26]. These conflicting results may stem from errors caused by the extreme inhomogeneity of these tumours due, in part, to large areas of necrotic tissue. These factors are less likely to be prominent in the smaller, more homogeneous rat tumours; a high correlation was noted here between tumour weight and urinary metabolite output values. The lack of correlation between tumour weight and HMPG output is difficult to explain although it is consistent with the absence of correlation between urinary HMPG and noradrenaline excretion. Dopamine output, on the other hand, correlates highly with that of its alcoholic metabolite, HMPE. Inaccuracies in determining human tumour weights might also account for the large variation previously noted in catecholamine turnover rates [19, 23]. In the three rats where tumour dopamine levels were measured, turnover rates for dopamine were relatively constant. The values were of the same order as those obtained for noradrenaline and adrenaline turnover in human tumours [27].

Table 3. Catecholamine content and dopamine turnover in tumour tissue

Rat	Tumour weight (g)	Noradrenaline (nmol/mg protein)	Adrenaline	Dopamine	Dopamine turnover rate (%)
4	2.1	23			
5	1.8	49			
6	1.3	20			
7	1.9	16			
8	6.7	9			
9	1.1	33			
10	1.4	29*	0.47*	1.47*	1000
11	1.1	–			
12	0.42	–			
13	0.92	24*	0.38*	2.20*	780
14	1.2	30	0.53*	1.47*	830
15	1.5	–			
Mean ± S.E.M.	1.8 ± 0.46	25.9 ± 3.8	0.46 ± 0.04	1.71 ± 0.24	870 ± 67

Catecholamine values marked with asterisks were determined using a radioenzymatic method which involves methylation with (^{3}H)*S*-adenosylmethionine followed by acetylation and separation by TLC [11, 12]. The remaining catecholamine values were determined using a trihydroxyindole fluorimetric assay. Dopamine turnover is calculated as the percentage of dopamine and its metabolites excreted relative to their tumour levels. The morphology of the tumour in rat 8 was atypical.

The increased excretion of the methylated metabolites, HMPG, HMPE and HVA, in tumour-bearing rats must, in view of the absence of catechol *O*-methyltransferase in the tumour [4], indicate circulation of the amines and their metabolites to some organ (e.g. liver or kidney) which contains the enzyme. The observed differences in the dopamine secretion pattern observed here to those observed in the clinical situation suggests that the rat model for human phaeochromocytoma [28] may only be valid for certain aspects of catecholamine metabolism and secretion *in vivo*. Further studies are required to clarify the situation. The rat model, nonetheless, has several unique advantages which include tumour homogeneity, reproducible tumour incidence and ready access for experimental manipulation.

Acknowledgements—G. R. was supported by the Cancer Research Campaign and R. L. P. in part by research grant HL29025.

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