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# EXCRETION OF NOREPINEPHRINE AND DOPAMINE ALCOHOLIC METABOLITES AFTER 6-HYDROXYDOPAMINE

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**Abstract**—Urinary excretion of 4-hydroxy-3-methoxyphenylglycol (HMPG) and 4-hydroxy-3-methoxyphenylethanol (HMPE) sulfates (HMPG-$SO_4$ and HMPE-$SO_4$) and of their two glucuronides (HMPG-Gluc) and (HMPE-Gluc) were critically studied in normal and demedullated rats after 6-hydroxy-dopamine (6-HD) treatment. 6-HD hydrobromide (25 mg/kg) was administered either intravenously (chemical sympathectomy) or intraventricularly (central sympathectomy). Demedullation did not significantly change the output of any of the above conjugated metabolites. Chemical sympathectomy significantly reduced the excretion of HMPG-$SO_4$ by about 40 per cent. The other metabolites we measured were not changed by chemical sympathectomy. Evidence was provided that indicated the presence in the peripheral sympathetic nervous system of nerve terminals resistant to destruction by 6-HD. These resistant nerve terminals were further shown to be involved in the production of HMPG-Gluc. Central sympathectomy fails to change the urinary excretion of the metabolites studied, including HMPG sulfate or glucuronide.

6-HYDROXYDOPAMINE (6-HD) injected parenterally, selectively destroys catecholaminergic nerve terminals in periphery.[1–3] This action has been exploited as a tool in studies of catecholaminergic nerves. Although the long-lasting depletion of catecholamines by 6-HD was first reported 10 yr ago,[4] the phenomenon of nerve degeneration caused by this drug was not discovered until several years later.[2] The extent of nerve terminal degeneration caused by 6-HD is dependent on such factors as age, dosage[5] and route of drug administration.[2] Until recently, very little attention was paid to the quantitative changes in the excretion of catecholamine metabolites after intravenous or intraventricular administration of 6-HD to experimental animals. In part, this neglect was due to the legitimate assumption that a reduced tissue catecholamine content goes hand-in-hand with a reduced metabolite excretion. However, changes in the urinary output of catecholamine metabolites do not reflect exclusively the extent of denervation brought about by 6-HD because the neurons which remain intact may display a compensatory increase of catecholamine turnover rate.[6]

It is currently believed that the sulfate conjugation of 4-hydroxy-3-methoxyphenylglycol (HMPG-$SO_4$) is a major pathway for brain norepinephrine (NE) metabolism.[7–10] We report experiments designed to reveal whether 6-HD injected systemically or intraventricularly affects the 24-hr excretion of the sulfate and glucuronide conjugates of HMPG and 4-hydroxy-3-methoxy-phenylethanol (HMPE) in rat.

## METHODS AND MATERIALS

Three consecutive 24-hr urine specimens were collected from male, Sprague Dawley rats individually housed in a low temperature urine collection device described by Denckla.[11] Rats received 6-HD according to one of the following schedules:

(1) Six rats (schedule 1) were injected i.v. with 25 mg/kg of 6-HD hydrobromide (6-HD-HBr) dissolved in 0·3 M ascorbic acid in 0·9 per cent NaCl solution. This dose of 6-HD was repeated 1 week later.

(2) Six rats (schedule 2) were treated as above, but doses of 6-HD-HBr were 50 and 100 mg/kg respectively.

(3) Six rats (schedule 3) were injected i.v. with 50 mg/kg of 6-HD-HBr followed by another dose 9 hr later. A week later, two more doses of 100 mg/kg of 6-HD-HBr were injected within a 24-hr interval.

(4) Six demedullated rats (schedule 4) received 6-HD 14 days after surgery according to schedule 1; higher doses of 6-HD, e.g. 50 mg/kg, were found to be fatal.

(5) Six normal and six demedullated rats (schedule 5) were injected twice intraventricularly with 200 $\mu$g 6-HD-HBr through a cannula permanently implanted into the lateral ventricle. The time between the first and second injection was 5 days. An equal number of normal and demedullated rats with implanted cannulae were injected with saline and used as controls. Procedures employed in schedules 1, 2, 3 and 4 will be generally referred to in the text as chemical sympathectomy; the procedure in schedule 5 will be referred to as central sympathectomy.

The urines of the group of rats injected according to each of the above schedules were collected for at least 2 weeks after the last injection of 6-HD. These urines were analyzed for glucuronide (Gluc) and sulfate ($SO_4$) conjugates of HMPG and HMPE by a gas chromatographic method previously described.[12]

Rats receiving 6-HD according to schedule 2 together with normal rats of the same age and weight were killed and their brains, hearts, aortas, mesenteric arteries and adrenals removed and assayed for NE by the method of Neff *et al.*[13] The NE content of brains and hearts of rats injected intraventricularly with either 6-HD or saline were also assayed.

In an experiment designed to detect the origin of HMPG-Gluc, seven rats were injected subcutaneously with 400 $\mu$g/kg of NE suspended in peanut oil. Urines were collected for 24 hr 3 days earlier and for 24 hr post-injection.

## RESULTS

Demedullation failed to change the excretion pattern of HMPG and HMPE conjugates (Table 1). In contrast, pretreatment with 6-HD reduced the urinary excretion of HMPG-$SO_4$. However, the reduction of this metabolite excretion is similar in intact and demedullated-rats receiving 6-HD. The excretion of HMPG-Gluc, HMPE-$SO_4$ and HMPE-Gluc was not significantly altered (see Table 1). The patterns of HMPG and HMPE excretion in the three groups subjected to various treatment schedules with 6-HD (schedules 1–3) are compared in Table 2. Again, every group of animals receiving 6-HD excreted less HMPG-$SO_4$ than did normal intact rats. However, neither MHPG-Gluc nor the conjugated metabolites of HMPE were significantly changed in these three groups of rats.

A subcutaneous injection of NE (400 mg/kg) suspended in oil did not change the amounts of glucuronide and sulfate conjugates of HMPG excreted during 24 hr.

TABLE 1. EXCRETION OF HMPG AND HMPE CONJUGATES BEFORE AND AFTER i.v. 6-HD*

| Experimental | HMPG-$SO_4$ | HMPG-Gluc | HMPE-$SO_4$ | HMPE-Gluc |
|---|---|---|---|---|
| Normal (intact) | 60 ± 3 | 8·3 ± 0·9 | 2·3 ± 0·5 | 3·5 ± 0·7 |
| Demedullated | 56 ± 3·5 | 7·1 ± 0·9 | 4·0 ± 0·8 | 4·0 ± 0·4 |
| Normal 6-HD | 38 ± 2·0† | 6·6 ± 0·5 | 1·9 ± 0·4 | 3·3 ± 0·3 |
| Demedullated 6-HD | 38 ± 1·7† | 11·0 ± 1·2 | 4·3 ± 0·7 | 3·4 ± 0·6 |

* Metabolite excretion is expressed as μg/24 hr; each value is the mean of six determinations. Treated rats were injected twice i.v. with 6-HD-HBr (25 mg/kg) with a 1-week interval between the two injections (schedules 1 and 4). Urines were collected at least 2 weeks after the last injection.

† $P < 0.005$ when compared to untreated controls.

TABLE 2. EFFECT OF 6-HD ADMINISTERED IN THREE DOSE SCHEDULES ON THE EXCRETION OF HMPG AND HMPE CONJUGATES*

| Experimental | 6-HD dosage (i.v.) | HMPG-$SO_4$ | HMPG-Gluc | HMPE-$SO_4$ | HMPE-Gluc |
|---|---|---|---|---|---|
| Normal | None | 60 ± 3 | 6·2 ± 0·4 | 2·3 ± 0·5 | 3·5 ± 0·7 |
| Schedule 1 | 25 mg/kg given twice | 38 ± 2† | 6·6 ± 0·5 | 4·0 ± 0·8 | 4·0 ± 0·4 |
| Schedule 2 | 25 mg/kg and 50 mg/kg | 38 ± 1·9† | 8·5 ± 0·5 | 2·1 ± 0·4 | 3·3 ± 0·3 |
| Schedule 3 | 50 mg/kg twice and 100 mg/kg twice | 31 ± 2·5† | 12·0 ± 1·6 | 4·7 ± 0·6 | 3·4 ± 0·6 |

* Urinary excretion is expressed as μg/24 hr. Urines were collected 2 weeks after the last injection of 6-HD. Further details of the time schedule of 6-HD injection are reported in Methods. Each value is the mean of six determinations.

† $P < 0.005$ when compared to normal rats.

TABLE 3. NE CONCENTRATIONS IN TISSUE OF RATS RECEIVING 6-HD*

| Tissue | NE (μg/g) Control | NE (μg/g) 6-HD | Change (%) | P value |
|---|---|---|---|---|
| Heart† | 1 ± 0·03 | 0·40 ± 0·031 | 60 | <0·001 |
| Aorta | 1·1 | 0·61 | 53 | |
| Mesenteric artery‡ | 2·6 | 2·2 | 15 | |
| Adrenals† | 4·5 ± 0·35 | 4·5 ± 0·30 | 0 | NS |
| Brain† | 0·26 ± 0·023 | 0·28 + 0·027 | 0 | NS |

* Rats were injected with 50 mg/kg i.v. of 6-HD-HBr, followed a week later by another dose of 100 mg/kg (schedule 2). Animals were killed 3 weeks after the last injection of 6-HD. NS = not significant.

† The results are the means of four determinations.

‡ The results are from a single determination of pooled tissues from four rats.

TABLE 4. NE CONTENT IN BRAIN AND HEART OF RATS INJECTED INTRAVENTRICULARLY WITH 6-HD*

| Tissue | NE (μg/g) Control | NE (μg/g) 6-HD | Decrease (%) | P value |
|---|---|---|---|---|
| Brain | 0·29 ± 0·02 | 0·10 ± 0·004 | 66 | <0·001 |
| Heart | 0·99 ± 0·097 | 1·1 ± 0·047 | None | NS |

* 6-HD hydrobromide, 200 μg, was injected twice via permanently implanted cannulae. In the controls, 20 μl saline was injected. Results are the means of six determinations. NS = not significant.

TABLE 5. EXCRETION OF CONJUGATED METABOLITES OF HMPG AND HMPE AFTER INTRAVENTRICULAR INJECTION OF 6-HD (CENTRAL SYMPATHECTOMY)*

| Experimental | HMPG-$SO_4$ | HMPG-Gluc | HMPE-$SO_4$ | HMPE-Gluc |
|---|---|---|---|---|
| Central sympathectomy | 63·3 ± 6·3 | 10·5 ± 1·5 | 4·7 ± 0·6 | 4·8 ± 0·6 |
| Intraventricular saline | 63·5 ± 3·6 | 9·3 ± 3·2 | 7·5 ± 0·8 | 5·7 ± 0·7 |
| Demedullated central sympathectomy | 41·1 ± 2·8 | 8·7 ± 1·3 | 2·3 ± 0·3 | 3·5 ± 0·5 |
| Demedullated intraventricular saline | 46·8 ± 0·6 | 7·4 ± 0·6 | 2·8 ± 1·0 | 3·0 ± 0·3 |

* Urinary excretion of various metabolites is expressed as μg/24 hr. Central sympathectomy was performed by injecting intraventricularly 6-HD-HBr, 200 μg, twice at an interval of 5 days (schedule 5). Urines were collected at least 2 weeks after the last injection.

The mean S.E.M. ($n$ = 6) of HMPG-Gluc excretion before and after NE administration were 6·4 ± 0·6 and 6·1 ± 0·9 μg/24 hr respectively. The corresponding values for HMPG-$SO_4$ were 55·9 ± 3·1 and 43·9 ± 4·7 μg/24 hr. The NE content of brain, heart, adrenals, aorta and mesenteric arteries of normal and 6-HD-treated rats is reported in Table 3. The 6-HD injections depleted cardiac NE very effectively but they depleted by a lesser extent the NE stores of mesenteric arteries. The NE content of the brain and adrenals was not affected by this dose of 6-HD injected intravenously. The NE content of brain and heart of six rats injected intraventricularly with 6-HD is shown in Table 4. The cardiac content was unchanged, while the brain NE content was reduced by 66 per cent. Intraventricular administration of 6-HD to normal and demedullated rats did not significantly reduce the excretion of HMPG or HMPE conjugates (Table 5).

## DISCUSSION

In the rat and in a number of other species, MHPG is a major metabolite of NE synthesized in brain[7,14,15] and in periphery.[16,17] In man[15] and in the guinea pig,[18] the main metabolite of NE synthesized in periphery is vanylmandelic acid (VMA); this metabolite may be formed secondarily from HMPG.[19,20] Urinary HMPE is a minor metabolite of dopamine. In rat, HMPG and HMPE are excreted almost completely conjugated.[12] The present report shows that HMPG-$SO_4$ is a urinary metabolite of NE which decreases when peripheral noradrenergic nerve terminals are lesioned by 6-HD treatment. The reduction in the urinary excretion of HMPG-$SO_4$ elicited by chemical sympathectomy is comparable to that reported by Ceasar *et al.*[21] after immunosympathectomy.

The results presented suggest that in the rat the excretion of HMPG-$SO_4$ may be a sensitive index of the anatomic integrity of peripheral sympathetic nerves located outside the blood vessels. The insignificant change in the excretion of HMPG and HMPE glucuronide and sulfate conjugates after demedullation lends itself to two explanations. First, it is possible that under normal conditions the contribution of the rat adrenal medulla to the total excretion of these conjugates is small compared to the total daily output of these NE metabolites. If the amount of conjugated HMPG and HMPE contributed by the adrenals is smaller than the individual variability in the excretion of HMPG and HMPE, its detection will be difficult. Second, the increase of catecholamine synthesis in adrenergic nerves, compensatory to demedullation, may obliterate the decrease of catecholamine conjugated metabolites due to the

obliteration of adrenal medulla.[6] If the second alternative were true, then the neurons involved in this compensatory mechanism must be insensitive to the effects of 6-HD, since the excretion of HMPG and HMPE was not significantly different in normal and medullated rats receiving 6-HD (Table 1).

By a systematic process of elimination, we have arrived at the conclusion that HMPG-Gluc is neuronally produced in the peripheral nervous system. Neither feeding the rats with a synthetic casein diet[12] free from tyrosine* and dihydroxyphenylalanine (dopa)[22] nor intraventricular injections of 6-HD (Table 4) changed HMPG-Gluc excretion. The possibility that HMPG-Gluc could be derived from the metabolism of dietary catechols by liver was also dismissed. Moreover, a subcutaneous injection of 400 $\mu$g/kg of NE in oil failed to change the excretion of HMPG-Gluc. The NE storage sites responsible for the production of HMPG-Gluc must also be resistant to 6-HD destruction, since HMPG-Gluc excretion was not reduced by chemical sympathectomy.

While the extent of nerve terminal degeneration in heart and spleen depends on the dose of 6-HD administered,[2] the overall effect of HMPG-$SO_4$ produced by 6-HD doses greater than 25 mg/kg appears to be dose independent (Table 2). The continued excretion of HMPG-$SO_4$, even after a very high dose of 6-HD known to produce over 90 per cent reduction in the heart and spleen NE content,[2] strongly points to the presence in the peripheral sympathetic nervous system of neurons resistant to 6-HD destruction. Berkowitz *et al.*[23] recently arrived at a similar conclusion from experiments with rats and guinea pigs receiving 6-HD or being immunosympathectomized. They located this resistant pool of NE in the sympathetic nerves of the blood vessels. The adrenergic terminals in the intestine are also resistant to immunosympathectomy.[24] We have confirmed that the catecholamine content of mesenteric arteries is relatively resistant to depletion by 6-HD.

The large contribution of the peripheral sympathetic nervous system to the total excretion of HMPG-$SO_4$ may be a factor responsible for the failure to observe any significant change in the excretion of HMPG-$SO_4$ after central sympathectomy. Although intraventricular injection of 6-HD reduces the turnover rate of brain NE,[25] the excretion of HMPG-$SO_4$ was not reduced in rats centrally sympathectomized (Table 5). Thus, in rats it is not easy to evaluate the brain contribution of HMPG-$SO_4$ in the urine by measuring the change with time in its total excretion after intraventricular 6-HD. This approach may have some value in other animal species where the predominant peripheral NE metabolite is not HMPG-$SO_4$. In monkeys, Maas *et al.*[26] were able to detect a significant reduction in the HMPG output after intraventricular injection of 6-HD.

Our results demonstrating that intraventricular administration of 6-HD fails to change HMPG excretion agree with a report by Hoeldtke *et al.*[27] Breese *et al.*[28] reported a significant reduction in the total HMPG excretion after intraventricular administration of 6-HD to rats but not to monkeys. These authors injected 6-HD with an irreversible monoamine oxidase inhibitor and possibly they have obtained a more extensive destruction of noradrenergic neurons of the rat brain. However, methodological discrepancies cannot be ruled out because the 24-hr HMPG excretion reported by these workers[28] is more than twice that published by

* R. Hoeldtke and R. Wurtman, personal communication.

others.[12,21,29] Although there is a tendency for the 24-hr urinary excretion of HMPG to vary from one batch of rats to another,[12] neither in the present nor in those reported by others[21,29,30] has a urinary output of total HMPG greater than 80 μg/24 hr been observed.

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