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Effect of 6-hydroxydopamine on the metabolism of endogenous octopamines and catecholamines

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The daily excretion in rat urine of *o*-(OHMA) (0·2), *m*-(MHMA) (0·9), *p*-(PHMA) hydroxymandelic acid (78), vanillylmandelic acid (VMA) (3·5) and homovanillic acid (30 μg day^{-1}), terminal metabolites of the isomeric octopamines and catecholamines, has been determined by gas chromatography-mass spectrometry. Chemical sympathectomy with 6-hydroxydopamine (i.p.) caused the daily output of each acid (except OHMA) to drop by 50% and that of PHMA was restored to normal the most rapidly after cessation of treatment. These results indicate that *m*- and *p*- (but not *o*-) octopamine coexist with catecholamines in sympathetic nerve terminals, are subject to the same compensatory biosynthetic mechanism following chemical sympathectomy and that *p*-octopamine has the highest turnover rate of these biogenic amines in the rat.

p-Octopamine (*p*-hydroxyphenylethanolamine) occurs in several sympathetically innervated organs of the rat (Molinoff & Axelrod 1969). It is believed to be located in sympathetic nerve endings and to function as a cotransmitter with noradrenaline (Axelrod & Saavedra 1977). *m*-Octopamine has been demonstrated to occur naturally in brain (Danielson et al 1977) and in sympathetically innervated organs such as salivary gland (Robertson et al 1977; Williams & Couch 1978), heart, spleen and vas deferens (Williams et al 1984). It is likely that both *m*-octopamine and *p*-octopamine are present in sympathetic nerve terminals containing noradrenaline. 6-Hydroxydopamine causes long-lasting depletion of noradrenaline in sympathetically innervated organs (Porter et al 1963, 1965). This depletion causes acute degeneration of the adrenergic nerve terminals resulting in chemical sympathectomy (Tranzer & Thoenen 1968). 6-Hyroxydopamine also causes a marked decrease in *p*-octopamine concentration in sympathetically innervated organs of the rat (Molinoff & Axelrod 1969). If *m*-octopamine is located in the same sympathetic nerve terminals as *p*-octopamine and catecholamines, then the administration of 6-hydroxydopamine would similarly affect the concentrations of these amines and of their metabolites. The main terminal urinary metabolites of the octopamines and catecholamines are: *o*-hydroxymandelic acid (OHMA) (*o*-octopamine) (James et al 1983); *m*-hydroxymandelic acid (MHMA) (*m*-octopamine); *p*-hydroxymandelic acid (PHMA) (*p*-octopamine); vanillylmandelic acid (VMA) (adrenaline and noradrenaline); homovanillic acid (HVA) (dopamine). We have investigated the effects of the administration of 6-hydroxydopamine on the levels of these metabolites in rat urine.

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Method

Control urine was collected from each of 5 male Sprague-Dawley rats (200–300 g) for three successive 24 h periods. Freshly prepared 6-hydroxydopamine solution in 0·9% NaCl (saline) was then administered intraperitoneally (50 mg kg^{-1}) to each rat and urine was collected for the following 24 h. The injection was repeated and two successive 24 h urine specimens were collected.

The pH of each urine sample was adjusted to 1 with conc. HCl, the acidic metabolites were extracted with ethyl acetate (3 × 3 ml), and combined ethyl acetate extracts evaporated (under vacuum) to small volume, transferred to a small glass vial and the solvent removed with nitrogen. The dried residue was reacted with methanolic HCl (100 μl) at room temperature for 15 min and the solvents removed under nitrogen. The resultant residue, containing the dry methylated derivatives, was reacted with pentafluoropropionic anhydride (PFPA) at 60 °C for 15 min. Excess PFPA was removed (nitrogen) and the dried Me-PFP derivatives of the acids were examined by gas chromatography–mass spectrometry-selected ion monitoring.

Identification of the acid-Me-PFP derivatives was achieved by establishing that the retention times and the ratio of the intensities $M^+/(M\text{-}COOCH_3)^+$ obtained from the derivatized urinary extracts were identical to those given by the corresponding authentic standards. Quantitative measurement of the acids was by using deuterated analogues as internal standards ($[^2H_4]$OHMA, 1 μg; $[^2H_3]$MHMA, 1 μg; $[^2H_2]$PHMA, 50 μg; $[^2H_2]$HVA, 25 μg and $[^2H_1]$VMA, 5 μg (Ibrahim et al 1984).

Results and discussion

The effect of the administration of 6-hydroxydopamine on urinary concentrations of endogenous OHMA, MHMA, PHMA, HVA and VMA is plotted in Fig. 1. The average daily excretion of these acids in urine is OHMA 0·2 (±0·1), MHMA 0·9 (±0·2), PHMA 78 (±16), HVA 30 (±6) and VMA 3·5 (±0·7) μg. The concentration of PHMA (the terminal metabolite of *p*-octopamine) is approximately 2·5 times that of HVA (the metabolite of dopamine), 20 times that of VMA (the metabolite of adrenaline and noradrenaline) and 85 times that of MHMA (the metabolite of *m*-octopamine). This observation indicates that *p*-octopamine may have the highest turnover of these endogenous

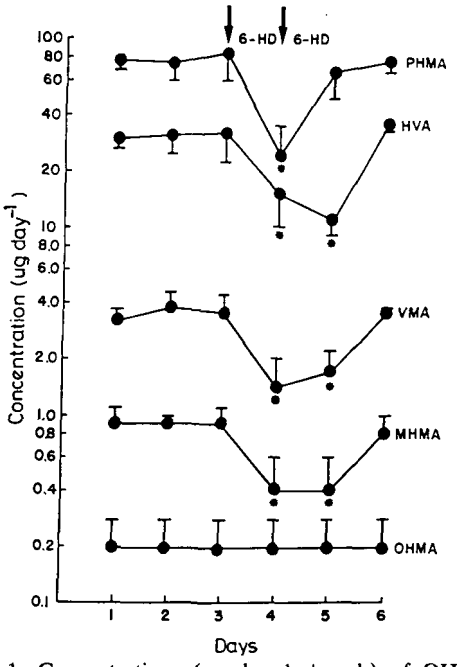

FIG. 1. Concentrations (μg day^{-1} ± s.d.) of OHMA, MHMA, PHMA, HVA and VMA in control rat urine (day 1, 2 and 3), 24 h after the first administration of 6-hydroxydopamine (day 4), 24 h after the second administration of 6-hydroxydopamine (day 5) and 48 h after the last of treatment. *$P > 0{\cdot}005$. 6-HD = 6-hydroxydopamine (50 mg kg^{-1}).

amines in the rat. This is consistent with the observation that the turnover of *p*-octopamine in the rat heart is six times faster than that of noradrenaline (Molinoff & Axelrod 1972). Except for OHMA, the concentrations of all the acids were decreased by approximately 50% after the administration of 6-hydroxydopamine indicating that the levels of endogenous *m*- and *p*-octopamine and the catecholamines were reduced to (at least) a similar extent. The fall of the urinary concentrations of MHMA, PHMA, VMA and HVA to only 50% is probably due to the fact that the adrenal glands are not affected by chemical sympathectomy (Mueller et al 1969). The concentration of PHMA was almost normal 24 h after the second administration of 6-hydroxydopamine while MHMA, HVA and VMA were still well below their normal urinary concentration levels. This is further evidence for the rapid metabolic turnover of *p*-octopamine. Concentrations of MHMA, PHMA, HVA and VMA were restored to normal 48 h after the last treatment with 6-hydroxydopamine. It is evident from these results that the concentration changes of these four acids in response to 6-hydroxydopamine are similar, providing additional evidence for the hypothesis that both *m*- and *p*-octopamine are synthesized and stored in sympathetic nerve endings. That *m*-octopamine normally coexists with *p*-actopamine in mammalian tissues innervated by sympathetic nerves (Williams et 1984) agrees with Reimann's (1984) finding that *m*-octopamine is taken up in noradrenergic nerve terminals, accumulates in the transmitter storage vesicles and is secreted with noradrenaline upon stimulation.

Although 6-hydroxydopamine has a profound effect upon the urinary concentrations of MHMA, PHMA, VMA and HVA, no effect on OHMA was observed. We interpret this finding to mean that *o*-octopamine is not localized in the same tissues (presumably sympathetic nerve terminals) that contain *m*- and *p*-octopamine and the catecholamines.

It has been reported that following the administration of 6-hydroxydopamine there is a compensatory mechanism whereby the adrenal medulla and autonomic (sympathetic) ganglia form more noradrenaline and hence restore its levels to normal (Mueller et al 1969). Our observation that the concentrations of HVA and VMA were restored to normal 48 h after the administration of 6-hydroxydopamine agrees with those findings. It can be seen (Fig. 1) that MHMA and PHMA behave in the same way as HVA and VMA. This indicates that the *m*- and *p*-octopamine are present in tissues, beside adrenergic neurons, such as the adrenal gland and autonomic ganglia and are affected by the same compensatory mechanism as noradrenaline. Furthermore, it appears that this compensation is more rapid in case of *p*-octopamine in which the concentration was restored to normal in a shorter (24 h) period.

Our results suggest that *m*- and *p*- but not *o*-, octopamine are present with catecholamines in sympathetic nerves. Moreoever, like catecholamines, *m*- and *p*-octopamine levels are restored by a compensatory mechanism following chemical sympathectomy.

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