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EFFECTS OF DEBRISOQUIN ON THE EXCRETION OF CATECHOLAMINE AND OCTOPAMINE METABOLITES IN THE RAT AND GUINEA PIG

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Abstract—The effects of debrisoquin, administered daily for 4 days to rats (40 mg/kg, i.p.) and guinea pigs (4 mg/kg, i.p.), were determined for urinary excretion of several acidic and neutral amine metabolites, including the norepinephrine metabolites, 3-methoxy-4-hydroxyphenethylene glycol (MHPG) and vanillylmandelic acid (VMA), the dopamine metabolites, 3,4-dihydroxyphenethanol (DHPE), 3-methoxy-4-hydroxyphenethanol (MHPE), and homovanillic acid (HVA), and the octopamine metabolite, *p*-hydroxyphenylglycol (pHPG). The excretion of MHPG was reduced to 32% of control in rats and to 46% in guinea pigs, HVA was reduced to 64 and 80% in these two species, respectively, and MHPE was lowered to 59% of control in the rat but was not affected in the guinea pig. DHPE and pHPG were not altered significantly in either species. VMA was a minor metabolite in both species, being less than 6% of MHPG, and its formation was blocked only partially (rat) or not at all (guinea pig) by debrisoquin. The data refute the idea based on previous *in vitro* studies that VMA is a major metabolite of norepinephrine in the periphery of the guinea pig as it is in man.

Debrisoquin is one of the class of drugs known as the adrenergic neuron blocking agents, which have antihypertensive properties [1]. Its mechanism of action has been suggested to be related to its ability to be selectively accumulated by adrenergic neurons, where it reaches sufficient concentrations to inhibit monoamine oxidase (MAO) [2–4]. Since the drug does not enter the brain [3], it has been used as a pharmacological tool for estimating the central and peripheral contributions to the urinary excretion of the norepinephrine (NE) metabolite, 3-methoxy-4-hydroxyphenethylene glycol (MHPG), in rats [5] and in man [6].

We have now examined the effects of debrisoquin in experimental animals on several of the acidic and neutral metabolites formed from dopamine (DA) and octopamine as well as from NE. We have included for comparison both rats and guinea pigs in our studies, since the guinea pig, like man but unlike the rat, is thought to synthesize vanillylmandelic acid (VMA) as the major metabolite of NE in the periphery [7, 8] (whereas MHPG is the principal metabolite in the CNS in each species). If correct, this would suggest that the guinea pig would be a good animal model for determining the origin of urinary metabolites in man.

MATERIALS AND METHODS

Animals and chemicals. Male Sprague–Dawley rats (240–250 g; Zivic–Miller, Allison Park, PA) and Hartley guinea pigs (225–250 g; Hilltop, Scottdale, PA) were housed in rooms with controlled temperature and lights on between 6:00 a.m. and 6:00 p.m. They were provided free access to a casein diet (No. 902487 for rats and No. 901911 for guinea pigs, ICN Nutritional Biochemicals, Cleveland, OH), since standard laboratory rat chows contain both DOPA and an enzyme that catalyzes the conversion of tyrosine to DOPA, thus elevating the urinary levels of catecholamine metabolites [9].

Homovanillic acid (HVA), DA, NE, epinephrine (EPI), 3,4-dihydroxybenzylamine and Type H-1 Helix pomatia sulfatase were obtained from Sigma (St. Louis, MO); VMA and acetyl chloride from Aldrich (Milwaukee, WI); 3-methoxy-4-hydroxyphenethanol (MHPE) and 3,4-dihydroxyphenethanol (DHPE) from Regis (Morton Grove, IL); 3-methoxy-4-hydroxyphenylpropionic acid (MHPPA) from ICN Pharmaceuticals (Plainview, NY); MHPG piperazine salt from Calbiochem-Behring (La Jolla, CA); pentafluoropropionic anhydride (PFPA) and pentafluoropropanol (PFPOH) from Pierce (Rockford, IL); octyl sodium sulfate from Eastman (Rochester, NY) and glass distilled methanol from Burdick & Jackson (Muskegon, MI). *p*-Hydroxyphenylglycol (pHPG) was synthesized by Dr. B. L. Goodwin (London) and 3-methoxy-4-hydroxyphenylpropanol (MHPP) was synthesized as previously described [10]. All other reagents were purchased from Fisher (Pittsburgh, PA). Debrisoquin sulfate (Ro 5-3307/1) was a gift of Dr. Peter Sorter, Hoffmann-La Roche (Nutley, NJ).

Experimental procedures. Animals were acclimated to metabolic cages (Maryland Plastics, Federalsburg, MD) and later injected daily with 40 mg/kg, i.p. (rats) or 4 mg/kg, i.p. (guinea pigs) of debrisoquin. Immediately following the fourth injection, the animals were again placed in metabolic cages and urines were collected for 24 hr into receptacles containing 5 mg of sodium metabisulfite. At the completion of each 24-hr urine collection, the animals were decapitated. The brains, hearts,

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Table 1. Effects of four daily injections of debrisoquin (40 mg/kg, i.p.) on tissue catecholamine concentrations in rats*

	Heart (ng/g)		Spleen (ng/g)		Adrenal glands (μg/g)		
Group	NE	EPI	NE	EPI	NE	EPI	DA
Saline	728 ± 36	61 ± 9	362 ± 59	23 ± 3	114 ± 10	412 ± 34	3.6 ± 0.3
Debrisoquin	286 ± 24 (39%) $P < 0.001$	52 ± 4	202 ± 32 (56%) $P < 0.05$	19 ± 1	108 ± 11	323 ± 44	7.4 ± 1.2 (206%) $P < 0.01$

* Values are the mean ± S.E.M. and were compared with two-tailed *t*-tests.

spleens and adrenal glands were rapidly removed, blotted, and frozen on dry ice. The urines were stored at −20° and the tissues were stored at −45° until assayed.

Biochemical assays. The neutral and acidic metabolites in urine and brain samples were assayed by chemical ionization gas chromatography–mass spectrometry (GC/MS), as previously described [10–12], except for VMA. The latter compound was derivatized to form the pentafluoropropionic methyl ester [13] and analyzed by GC/MS, using a 3% SP-2401 column operated isothermally at 160° and focusing on the base peaks *m/z* 341 ($MH^+ - PFPOH$) for VMA and *m/z* 325 ($MH^+ - CH_3OH$) for the internal standard, MHPPA. Tissue catecholamine levels were determined by high-performance liquid chromatography (HPLC) with electrochemical detection [14].

RESULTS

The effects of four daily injections of 40 mg/kg, i.p., of debrisoquin on catecholamine levels in three peripheral organs of rats are shown in Table 1. NE was lowered in the heart (to 39%, $P < 0.001$) and spleen (to 56%, $P < 0.05$) but was unaffected in the adrenal glands. DA was determined only in the adrenal glands, where it was elevated to 206% of control ($P < 0.01$). EPI was unaffected in each organ.

No effects of debrisoquin on amine metabolites in the brain were found. Brain MHPG levels were 133 ± 5 ng/g in debrisoquin-injected rats as compared to 123 ± 7 ng/g in saline-injected rats. Brain DHPE levels were 3.0 ± 0.3 ng/g and 2.9 ± 0.5 ng/g in the experimental and control groups, respectively, and brain pHPG levels were 1.5 ± 0.1 ng/g and 1.7 ± 0.2 ng/g in these groups.

In a pilot study, 2/2 guinea pigs died after receiving a single injection of 40 mg/kg, i.p., of debrisoquin. With a dose of 4 mg/kg, i.p., daily for 4 days, all of the animals survived and displayed no adverse effects. Table 2 shows that debrisoquin produced a similar but slightly greater reduction in NE in the heart (to 30%, $P < 0.001$) and spleen (to 31%, $P < 0.001$) than in the rat, even though the dose was only one-tenth as large. As in the rat, adrenal NE and EPI levels were unchanged, but in the guinea pig adrenal DA was undetectable.

The effect of the debrisoquin treatment on the excretion of urinary metabolites in the rat and guinea pig are shown in Figs. 1 and 2 respectively. In the rat, MHPG excretion was reduced to 32% ($P < 0.005$). There were also smaller but highly significant decreases in the excretion of free and total HVA (to 66 and 64% of control, $P < 0.005$). Similar decreases to 59% were observed for both of the neutral DA metabolites but only the decrease for MHPE was statistically significant ($P < 0.05$). The excretion of pHPG was 30% lower in the debrisoquin-treated rats, but this was not statistically significant.

The excretion of total MHPG was lowered to 46% ($P < 0.005$) in guinea pigs treated with debrisoquin (Fig. 2). Total HVA was lowered to 80% ($P < 0.05$). The neutral DA metabolites as well as pHPG were not statistically affected, with total MHPE being 59%; total DHPE, 104%; and total pHPG, 90% of control.

The concentrations of free MHPG and pHPG were also determined in guinea pig urine. In the control group, the excretion of free MHPG was 3.8 ± 1.0 μg/24 hr and that of free pHPG was 202 ± 16 ng/24 hr. Thus, about 95% of the total MHPG and 90% of the total pHPG were excreted in the conjugated form in this species. This is similar to the degree of conjugation of these metabolites in

Table 2. Effects of four daily injections of debrisoquin (4 mg/kg, i.p.) on tissue catecholamine concentrations in guinea pigs*

	Heart (ng/g)	Spleen (ng/g)	Adrenal glands (μg/g)	
Group	NE	NE	NE	EPI
Saline	1860 ± 30	3360 ± 130	22.1 ± 1.1	352 ± 30
Debrisoquin	550 ± 50 (30%) $P < 0.001$	1040 ± 90 (31%) $P < 0.001$)	17.5 ± 2.2	279 ± 39

* Values are the mean ± S.E.M. and were compared with two-tailed *t*-tests.

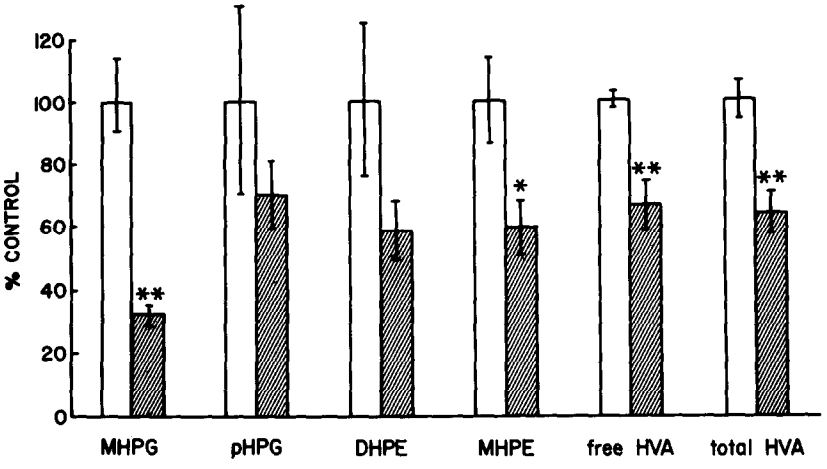

Fig. 1. Effects of debrisoquin (40 mg/kg, i.p., daily for 4 days) on the excretion of urinary metabolites in the rat. Values are shown as percent of controls. Control values in μg/24 hr (mean ± S.E.M.) were 25.7 ± 3.7 for MHPG, 2.7 ± 0.8 for pHPG, 1.9 ± 0.5 for DHPE, 0.95 ± 0.13 for MHPE, 20.7 ± 0.6 for free HVA, and 26.8 ± 1.6 for total HVA. Key: (□) controls (N = 6), and (■) debrisoquin-treated rats (N = 6); (*) $P < 0.05$, and (**) $P < 0.005$ (Student's two-tailed t-test).

rat urine, in which 92% of the total MHPG and 96% of the total pHPG were conjugated [15]. In guinea pigs treated with debrisoquin, the excretion of free MHPG was 2.9 ± 0.68 μg/24 hr, or 76% of control; but this decrease was not statistically significant. The urinary excretion of free pHPG in the debrisoquin-treated group was 197 ± 26 ng/24 hr and was virtually the same as the control group (98%).

Debrisoquin lowered total VMA excretion in rats to 53% of controls, from 0.65 ± 0.09 to 0.34 ± 0.07 μg/24 hr ($P < 0.05$). In contrast, VMA excretion in guinea pigs was unaffected, being 4.8 ± 1.2 μg/24 hr in control animals and 6.4 ± 0.8 μg/24 hr in debrisoquin-treated animals.

DISCUSSION

The ability of debrisoquin to selectively block the deamination of amines in the periphery suggests the possibility of estimating the proportion of endogenous amine metabolites that originates in the CNS by measuring the effects of debrisoquin on their excretion. The validity of this method is based on the assumptions that debrisoquin (1) completely blocks metabolite formation in the periphery, and (2) does not indirectly influence the rate of metabolism of neurotransmitter amines in the brain, even though the drug itself does not cross the blood–brain barrier. Unfortunately, the extent of *in vivo* MAO inhibition

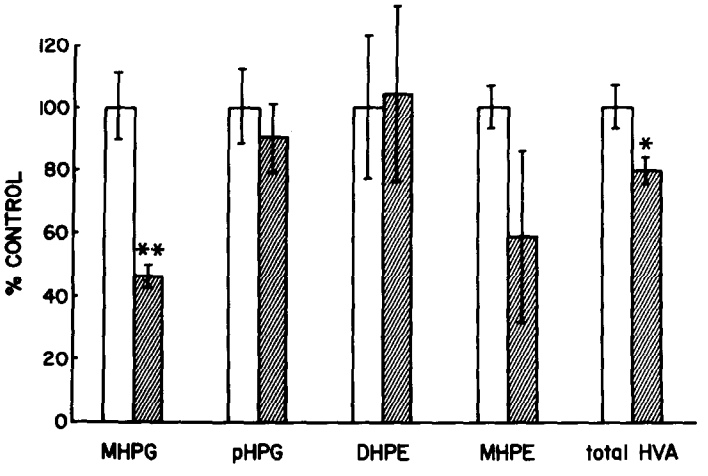

Fig. 2. Effects of debrisoquin (4 mg/kg, i.p., daily for 4 days) on the excretion of urinary metabolites in the guinea pig. Values are shown as percent of controls. Control values in μg/24 hr (mean ± S.E.M.) were 78.8 ± 8.4 for MHPG, 2.0 ± 0.2 for pHPG, 10.3 ± 2.4 for DHPE, 0.91 ± 0.25 for MHPE, and 43.5 ± 3.2 for total HVA. Key: (□) controls (N = 5), and (■) debrisoquin-treated rats (N = 5); (*) $P < 0.05$ and (**) $P < 0.005$ (Student's two-tailed t-test).

cannot be readily assessed because of the reversible nature of the inhibition. Although debrisoquin is a relatively weak MAO inhibitor *in vitro*, with an I_{50} of 2 μM, the drug is readily accumulated in tissues, reaching a tissue/medium concentration of greater than 10 in slice preparations [3]. Since uptake of the drug is inhibited in the presence of desipramine or amphetamine, it was suggested that the drug is selectively concentrated within neurons, presumably reaching a sufficient level to inhibit MAO [3]. This has been confirmed by fluorescence histochemistry for adrenergic neurons in the rat iris, where debrisoquin was ten times more potent than nialamide as an inhibitor of MAO [16]. Intraneuronal accumulation of debrisoquin could also explain the long duration of the antihypertensive effect and the presence of the drug in tissues for up to 16 hr after administration, despite a plasma half-life of only about 40 min [3].

That low doses of debrisoquin at least partially block peripheral MAO *in vivo* is evident by the finding that a dose schedule of 0.5 to 1.0 mg/kg per day reduces the peripheral contribution to plasma HVA [17]. A much higher dose of debrisoquin was employed in our study in order to inhibit MAO in the periphery as completely as possible. Although at high doses, debrisoquin has a norepinephrine-depleting action [18, 19], this should have no consequence in levels of deaminated metabolites, provided that MAO is inhibited completely. On the other hand, we cannot rule out the possibility that, in spite of the high dose regimen of debrisoquin used, a small amount of deamination persists in the periphery and that this could cause some overestimation of the contribution of the brain to urinary metabolites. This overestimation might be further augmented, if there were a compensatory increase in catecholamine synthesis in response to the amine-depleting action of the drug.

With regard to the assumption that debrisoquin has no indirect effect on brain NE metabolism, this is strongly supported by our finding that rat brain MHPG levels were unaltered by debrisoquin. This is in agreement with a previous report in the rat [20] but contrasts with claims that the drug decreases the production of MHPG (but not HVA) in the brain of monkeys (by 66%) [21] and of humans (by 57%) [22]. This discrepancy might be due to species differences.

If the above two assumptions are correct, we can calculate that about 32% of the urinary MHPG derives from the CNS. While we cannot be certain that MAO in the periphery is completely inhibited, it is interesting to note that Karoum *et al.* [5] obtained a similar estimate (30%), even though they administered debrisoquin as a single, low dose and corrected for any lack of complete blockade in deamination by measuring the excretion of tritiated metabolites following prelabeling of peripheral NE stores with an intravenous injection of [^{3}H]NE. These results are also consistent with the 36% calculated from data obtained 7 days after an intraventricular injection of 6-hydroxydopamine (6-OHDA) [23].

It should be noted that the rate of MHPG excretion found in our studies (25.7 μg/24 hr) was somewhat lower than that reported by Karoum *et al.* [5] (58 μg/24 hr) as well as by Kopin and Weise [24] (62 μg/24 hr). We have found very similar values in our laboratory for rats fed a standard lab chow (i.e. 60 μg/24 hr [11] and 54 μg/24 hr [25]) but have noted that MHPG excretion is 33% lower in rats maintained for 7 days on a casein diet [25]. Weight of the animals is also an important factor in the amount of MHPG excreted [11] and may account for some of the discrepancies among studies.

A comparison between the effects of debrisoquin on the excretion of MHPG and metabolites of DA and octopamine revealed that none of the latter metabolites was as greatly affected as was MHPG, suggesting that even higher proportions of them originate from the brain. For example, based on our results it is calculated that 66% of free HVA and 64% of total HVA derive from the CNS. This is somewhat higher than the estimates of 39 and 46%, respectively, obtained in rats treated centrally with 6-OHDA [23]. The higher estimates in the present study could be due to MAO activity in the periphery not being completely inhibited by the debrisoquin treatment.

We have suggested previously that the neutral DA metabolites, DHPE and MHPE, might be more selective markers of CNS DA metabolism than the acidic ones, even though they are relatively minor metabolites [11]. However, the present data indicate that this does not apply in the rat, since debrisoquin reduced their urinary levels to about the same extent as HVA. This is not surprising, since if, as previously mentioned, a large proportion of MHPG in the rat derives from the periphery, considerable amounts of other neutral metabolites, including those from DA, could also be synthesized there. On the other hand, in species such as man in which VMA is the predominate metabolite of NE in the periphery and MHPG is more selectively formed in the CNS, one would predict that neutral DA metabolites would likewise be more selectively synthesized in the CNS.

Our finding that debrisoquin failed to significantly lower pHPG excretion suggests that this metabolite may also derive largely from the CNS. If so, urinary pHPG levels would provide a useful marker of brain octopamine metabolism, provided that dietary sources of pHPG are eliminated, either by the use of a casein diet in animal studies [25] or by a VMA exclusion diet in human subjects [11]. On the other hand, we cannot presently rule out the possibility that pHPG was formed in the periphery, either at extraneuronal sites or in neurons that do not accumulate debrisoquin.

The effects of debrisoquin were studied in the guinea pig, since VMA is considered to be the main metabolite of NE in the periphery of this species as in man [7, 8]. Although the decline in MHPG excretion caused by debrisoquin was smaller in the guinea pig than in the rat ($P < 0.05$, *t*-test), the results nevertheless indicate that at least one-half of the total MHPG excreted originates in the periphery. On the other hand, VMA excretion in the guinea pig was only about 4.8 μg/24 hr, or about 6% of the MHPG. By contrast, normal adult men excrete in the range of 1.7 to 6.3 mg/24 hr [26], an amount that is about 2.2-fold higher than in the level of MHPG excretion [27]. These results taken together make it

clear that VMA is not the chief metabolite of NE in the periphery of the guinea pig *in vivo*. Thus, the guinea pig apparently resembles more closely the rat than man in the way NE is metabolized in the periphery.

Even the small amounts of VMA excreted by the rat and guinea pig may partially derive from a source other than from the direct conversion of NE to VMA. This is suggested by the finding that debrisoquin had a smaller effect on VMA than on MHPG in the rat and had no effect on VMA in the guinea pig. One possible source of VMA is MHPG, since it has been demonstrated recently that a significant amount of deuterated MHPG injected i.v. into man is converted to VMA [28, 29]. This pathway does not appear to be very significant in the rat or guinea pig, since the amount of total VMA excreted by these species is only 5–6% of total MHPG. Nevertheless, only a small degree of conversion of MHPG to VMA would be needed to produce all or part of the latter metabolite. This would account for the continued production of VMA during debrisoquin treatment, since MHPG originating in the CNS could continue to be converted to VMA. Alternatively, debrisoquin might enhance the conversion of MHPG to VMA, perhaps by blocking MHPG conjugation, and thus overcome any decrease due to debrisoquin in the direct production of VMA from NE in the periphery. Such a possibility seems unlikely, however, since the excretion of free MHPG was decreased by debrisoquin, and was not increased as would be expected if MHPG conjugation were blocked.

Since previous studies had shown that *p*-hydroxymandelic acid, the acidic metabolite of octopamine, could be formed as a degradation product of *p*-hydroxyphenylpyruvate [30], we next considered the possibility that VMA might be analogously produced as an artifact from 3-methoxy-4-hydroxyphenylpyruvate. When the latter compound was carried through our extraction scheme, we detected small amounts of VMA (about 0.4% of the added compound), which was identified by both GC/MS and HPLC (D. J. Edwards and J. Ravitch, unpublished observation). Since large quantities of 3-methoxy-4-hydroxyphenylpyruvate (532–945 μg/g creatinine) are excreted in urine [31], it is conceivable that all or part of the VMA in rat and guinea pig urine might originate in this way. In further support of this idea, we have also observed that VMA excretion was elevated by 2.3-fold ($P < 0.001$) in rats given a single injection of FLA-63 (25 mg/kg, i.p.), a dopamine-β-hydroxylase inhibitor (D. J. Edwards and J. Ravitch, unpublished observation). This is contrary to an expected decrease in VMA excretion if it were a metabolite of NE but is consistent with VMA deriving from a DA metabolite. Interestingly, the highest VMA levels in the rat brain are found in the DA-rich striatum [32]. Taken together, these results preclude the use of VMA excretion in the rat or guinea pig as a marker of peripheral NE metabolism and of *in vivo* MAO activity.

An interesting difference between the rat and guinea pig is that the latter requires much smaller doses of debrisoquin to produce the same effect. For example, the 40 mg/kg dose used in the rat, which was well below the LD_{50} for that species, was lethal to guinea pigs. Moreover, treatment of guinea pigs with 4 mg/kg for 4 days produced greater reductions in the NE content of the heart and spleen (to 30 and 31% of control, respectively) than did a 10-fold higher dose in the rat. By comparison, the dose of 40 mg/kg (4 days) in rats reduced NE in the heart to 39% and in the spleen to 56%. Similar decreases (to 42 and 56% respectively) have been found in rats treated for 11 weeks at a dose up to 110 mg/kg [19]. Although, as in the rat, we cannot say to what extent intraneuronal MAO is inhibited by our regimen of debrisoquin, it seems probable based on the above results that MAO is inhibited at least as effectively in the guinea pigs as in the rats.

An unexpected finding in these studies was that debrisoquin elevated the level of DA in the rat adrenal, whereas in agreement with a previous report [19], the levels of NE and EPI were unchanged. These findings could be explained by an inhibition of dopamine-β-hydroxylase, since the inhibitor FLA-63 similarly elevates adrenal DA levels without affecting NE and EPI [33]. However, this seems unlikely, since long-term treatment with debrisoquin has been found to stimulate rather than inhibit dopamine-β-hydroxylase activity [19]. A second possibility is that the increase in DA represents an acceleration in catecholamine synthesis resulting from catecholamine depletion. On the other hand, the MAO inhibitor pargyline has been found to similarly increase adrenal DA levels without affecting NE and EPI, although this treatment presumably does not increase catecholamine synthesis [33], while reserpine, which increases adrenal tyrosine hydroxylase activity and hence catecholamine synthesis [34], decreases adrenal DA levels [33]. Alternatively, these results may be related to recent evidence suggesting that DA is stored in the rat adrenal independently from other catecholamines [35].

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