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Inhibition of Human Brain Type B Monoamine Oxidase by Tricyclic Psychoactive Drugs

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SUMMARY

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The ability of a variety of tricyclic psychoactive drugs to inhibit human brain mitochondrial type B monoamine oxidase as measured by phenylethylamine (PEA) deamination was examined *in vitro*. At 50 μM all drugs tested, with the exception of chlorpromazine sulfoxide and imipramine *N*-oxide, inhibited this reaction between 40.8% and 78.4%. Lineweaver-Burk plots for imipramine, chlorpromazine, and chlorprothixene inhibition of PEA deamination displayed a mixed inhibition pattern when incubations were performed at normal atmospheric oxygen tension. When the oxygen concentration was elevated, inhibition of this reaction by each of the three drugs became more competitive. These results suggest that these drugs inhibit the B form of monoamine oxidase by binding to both the oxidized and reduced forms of the enzyme. Inhibition of monoamine oxidase by imipramine and desmethylimipramine increased as the pH was raised from 7.0 to 9.0, but because the ratio of the increase remained constant for the two drugs, inhibition probably was independent of the degree of ionization of the side chain aliphatic amine. It was also found that the optimal pH for human brain mitochondrial deamination of PEA shifted from 8.0 to 8.5 as the oxygen concentration was increased.

INTRODUCTION

In previous studies from this laboratory it was demonstrated that tricyclic antidepressant drugs inhibit both the A and B forms of rabbit brain mitochondrial monoamine oxidase (1-4). The B form of the oxidase, as measured by phenylethylamine deamination, was more sensitive than the A form to all tricyclic drugs tested, and the magnitude of inhibition was independent of the degree of ionization or basicity of the aliphatic amine side chain. It was concluded that the structure of the tricyclic ring moiety was the determining factor in the ability of these drugs to inhibit either form of the oxidase. In addition, the antidepressant agents iprindole and imipramine produced equal inhibition of the B form of rabbit monoamine oxidase (5). Since iprindole apparently does not prevent neuronal reuptake of catechol or indole amines (6-9), it was suggested that its ability to inhibit monoamine oxidase may take on a more significant meaning in regard to the mechanism of action of this antidepressant drug.

Edwards and co-workers (10, 11) have

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reported that tricyclic antidepressant drugs, including iprindole, also inhibit human platelet monoamine oxidase, the properties of which resemble the B form found in human brain tissue (12). They found that amitriptyline inhibits PEA[1] deamination noncompetitively, tryptamine degradation in a mixed fashion, and benzylamine oxidation competitively. This antidepressant drug also inhibited the B form of human brain mitochondrial monoamine oxidase in a similar manner when incubations were performed at normal atmospheric oxygen tension. However, when the concentration of oxygen was elevated during incubations with human brain monoamine oxidase, amitriptyline inhibited PEA deamination competitively (12). Since the monoamine oxidase reaction proceeds via a ping-pong mechanism (13, 14), it was concluded that amitriptyline probably inhibits monoamine oxidase activity by binding to both the oxidized and reduced forms of the brain enzyme (12).

The purpose of the present investigation was to determine whether other tricyclic psychomimetic agents also inhibit human brain monoamine oxidase deamination of PEA and to examine whether increased oxygen levels and pH alter the kinetics of inhibition by these clinically important drugs.

MATERIALS AND METHODS

For the experiments described in this paper, mitochondria were isolated from frontal lobes of human brain within 24 hr after death. Methods used to isolate mitochondrial monoamine oxidase and assay of the enzyme activity at normal and elevated oxygen concentrations have been described previously (12). In some cases the enzyme reactions were terminated by the addition of 0.2 ml of 0.4 M HCl instead of 0.2 ml each of 0.25 M $ZnSO_4$ and 0.20 M $Ba(OH)_2$. Deaminated products formed in the reactions were separated by cation-exchange chromatography (Bio-Rex 70) as reported previously (12). The A form of monoamine oxidase appears to be the more unstable of the two isoenzymes, and several mitochondrial preparations were found to be totally lacking in type A activity as measured by 5-hydroxytryptamine deamination. The kinetic properties of the B form of the enzyme were not affected by the length of time after death when brain specimens were obtained.

To determine the pH optima for human brain monoamine oxidase deamination of PEA in the presence and absence of tricyclic drugs and at normal and elevated oxygen tensions, incubations were performed as described above except that Tris-HCl buffer was used at a final concentration of 0.04 M instead of phosphate buffer. Since the enzyme stock solution was stored frozen in 0.1 M potassium phosphate buffer, pH 7.4, this solution was diluted, depending on the enzyme preparation, from 1:15 to 1:60 with 0.01 M phosphate buffer, pH 7.4, prior to use in these experiments. To initiate the reactions, a 0.2-ml aliquot of this diluted enzyme preparation was added to the reaction mixture, whose total volume was 2 ml. Addition of the enzyme suspension did not alter the final pH of the reaction mixture.

[^{14}C]Phenylethylamine (50.98 mCi/mmole) was purchased from New England Nuclear. The tricyclic drugs used in this study were gifts from Ciba-Geigy (imipramine, imipramine *N*-oxide, didesmethylimipramine, and chlorimipramine), USV (desmethylimipramine), Merck Sharp & Dohme (amitriptyline and protriptyline), Eli Lilly (nortriptyline), Hoffmann-La Roche (cyclobenzaprine and chlorprothixene), Charles Pfizer (doxepin), and Smith Kline & French (chlorpromazine and chlorpromazine sulfoxide). 7-Hydroxychlorpromazine was a gift from Drs. Robert Roth and B. S. Bunney of Yale University School of Medicine.

RESULTS

The ability of imipramine, amitriptyline, chlorpromazine, and chlorprothixene to inhibit human brain monoamine oxidase deamination of PEA is illustrated in Fig. 1. Amitriptyline and chlorprothixene were the most effective inhibitors of this

[1] The abbreviation used is: PEA, phenylethylamine.

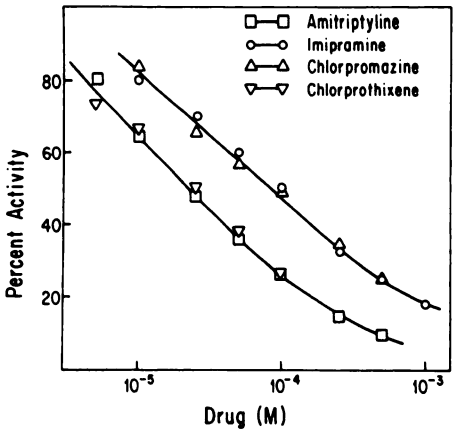

FIG. 1. *Effects of amitriptyline, chlorprothixene, chlorpromazine, and imipramine on human brain monoamine oxidase deamination of phenylethylamine*

Reaction mixtures containing 7.3–8.6 nmoles of [^{14}C]PEA, 0.10 mg–0.26 mg of mitochondrial protein, and various amounts of the tricyclic drugs in a total of 2 ml of 0.05 M potassium phosphate buffer, pH 7.4, were incubated for 10 min at 37°. In the absence of drug, 0.32–2.3 nmoles of deaminated product were formed, depending on the enzyme concentration and preparation used. Values shown are the averages of two experiments, each performed in duplicate.

reaction, both producing 50% reduction in activity at approximately 25 μM, and 20% inhibition at 4 μM. Imipramine and chlorpromazine, in contrast, inhibited PEA oxidation 20% and 50% at around 10 μM and 90 μM, respectively. In addition to the above tricyclic drugs, other iminodibenzyl, dibenzocycloheptene, and phenothiazine analogues were tested for inhibition of PEA deamination (Table 1). Of all the drugs examined, only imipramine *N*-oxide and chlorpromazine sulfoxide failed to inhibit monoamine oxidase significantly. Inhibition of PEA deamination ranged from 40.8% for desmethylimipramine to 78.4% for cyclobenzaprine. In general, the dibenzocycloheptene derivatives were the most effective inhibitors of this reaction, whereas the iminodibenzyl and phenothiazine derivatives were essentially equally potent against the B form of monoamine oxidase. The mono-*N*-demethylated products of imipramine and amitriptyline were only slightly less effective inhibitors of PEA deamination than the parent drugs. However, modification of the center ring structure of amitriptyline, as in protriptyline and doxepin, resulted in decreased inhibition of this reaction. In contrast, addition of a double bond to the ring system of amitriptyline, to produce cyclobenzaprine, caused an increase in the degree of inhibition of PEA degradation.

In addition to the drugs listed in Table 1, the effects of iprindole and opipramol on PEA deamination were examined (Fig. 2). Fifty per cent inhibition of this reaction was achieved at drug concentrations of 60 and 40 μM, respectively.

The type of inhibition of PEA deamination by imipramine, chlorpromazine, and chlorprothixene in the presence of limiting and high oxygen levels is illustrated by the Lineweaver-Burk plots in Fig. 3. At normal atmospheric oxygen tension all three drugs displayed a mixed inhibition pattern. Of the three drugs tested, chlor-

TABLE 1

Tricyclic drug inhibition of phenylethylamine deamination by human brain monoamine oxidase

Reaction mixtures containing tricyclic drug, 6.2 nmoles of [^{14}C]PEA, and 0.27–0.38 mg of mitochondrial protein were incubated for 10 min at 37° in 2 ml of 0.05 M potassium phosphate buffer, pH 7.4. In the absence of drug 1.2–2.0 nmoles of deaminated product were formed, depending on the enzyme concentration and preparation used. Values reported are the means ± standard errors of three experiments, each performed in duplicate.

Drug (50 μM)	Inhibition
	%
Dibenzocycloheptene analogues	
Amitriptyline	66.9 ± 0.9
Nortriptyline	58.1 ± 1.9
Protriptyline	49.8 ± 1.7
Cyclobenzaprine	78.4 ± 0.6
Doxepin	46.3 ± 0.9
Iminodibenzyl analogues	
Imipramine	45.0 ± 0.9
Desmethylimipramine	40.8 ± 0.7
Didesmethylimipramine	47.4 ± 2.4
Chlorimipramine	40.3 ± 1.7
Imipramine *N*-oxide	6.2 ± 4.4
Phenothiazine analogues	
Chlorpromazine	44.3 ± 0.4
7-Hydroxychlorpromazine	52.3 ± 1.6
Chlorpromazine sulfoxide	1.7 ± 1.7
Chlorprothixene	59.8 ± 2.5

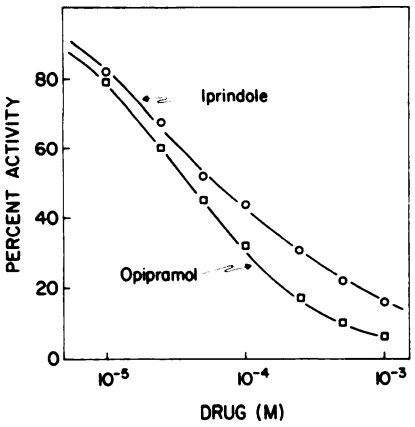

FIG. 2. *Effects of iprindole and opipramol on human brain monoamine oxidase deamination of phenylethylamine*

Reaction conditions are described in the legend to Fig. 1. Values shown are the averages of two experiments, each performed in duplicate.

promazine inhibition of monoamine oxidase most closely resembled uncompetitive kinetics. In similar experiments performed at elevated oxygen concentrations with all three tricyclic drugs, the Lineweaver-Burk plots shifted toward a more competitive inhibition pattern, although all still displayed mixed inhibition kinetics. These results are similar to those previously reported for amitriptyline inhibition of PEA deamination by human brain (12). Figure 3 also indicates that, of the three drugs tested at high oxygen tension, chlorprothixene most nearly resembled an apparent true competitive inhibitor of PEA deamination.

The above results suggest that the kinetics of inhibition of PEA deamination by tricyclic drugs is dependent on the concentration of oxygen used. To examine this effect of oxygen in greater detail, inhibition of PEA deamination by imipramine was determined at various pH values and at normal and elevated oxygen levels. The pH optimum for human brain deamination of PEA shifted to a higher value for incubations performed at high oxygen levels (Fig. 4). In the absence of imipramine a shift from pH 8.0 to 8.5 was observed, and in the presence of the inhibitor a shift from pH 7.5 to 8.0 or 8.5 was seen. These data also demonstrate a slight downward shift in pH optima from 8.0 to 7.5 and from 8.5 to approximately 8.0 for reactions performed in the presence of the tricyclic drug at both normal and elevated oxygen levels, respectively.

The percentage inhibition of PEA deamination by imipramine for the data shown in Fig. 4 is presented in Table 2. These data indicate that the magnitude of imipramine inhibition of monoamine oxidase steadily increased as the pH was raised for incubations performed at either oxygen concentration. For both conditions, percentage inhibition reached a plateau between pH 8.0 and 8.5, although this plateau was more pronounced for incubations performed at normal oxygen levels. At high oxygen tension the extent of inhibition at each pH value decreased slightly from that at low oxygen levels.

The observed increase in the magnitude

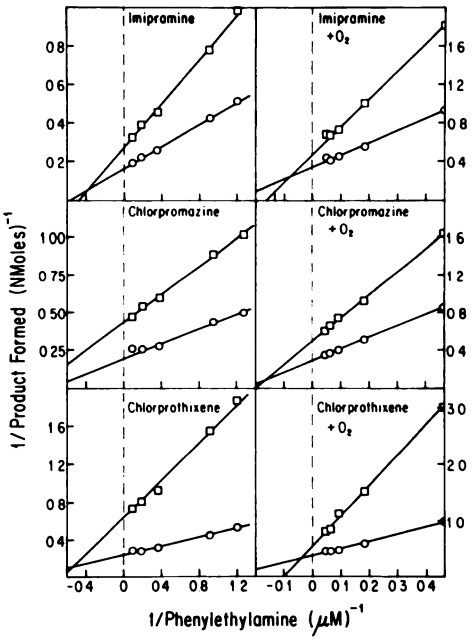

FIG. 3. *Lineweaver-Burk plots for inhibition of phenylethylamine deamination by imipramine, chlorpromazine, and chlorprothixene*

Various amounts of [^{14}C]PEA were incubated in the absence (O——O) and presence (□——□) of 50 μM tricyclic drug at normal and high oxygen tension at 37° for 10 min. Data shown are from a typical experiment. Each experimental point represents the average of duplicate determinations. All experiments were performed at least three times.

of inhibition of PEA deamination by imipramine as the pH was raised may be a result of increased formation of the un-ionized species of the drug. In order to determine whether the un-ionized form of imipramine is actually the biochemically active agent involved in inhibition, experiments were performed to compare the ability of imipramine (pK_a = 8.4) and desmethylimipramine (pK_a 9.5) to inhibit PEA deamination at pH 7.5 and 9.0. These structural analogues were chosen because their pK_a values differ such that at pH 9.0 the concentration of the un-ionized form of imipramine will be approximately twice that of its *N*-demethylated derivative. Both drugs produced a significant ($p < 0.005$) increase in percentage inhibition of PEA deamination at normal oxygen tension at pH 9.0 compared with pH 7.5 (Table 3). Although not statistically significant, this trend was also seen for incubations performed at elevated oxygen levels. This increase was observed at both normal and high oxygen concentrations. It can also be seen that the ratios of percentage inhibition for reactions inhibited by imipramine and desmethylimipramine at pH 7.5 and 9.0 were essentially identical: 0.81 compared with 0.78, and 0.50 compared with 0.53.

Experiments were also carried out to compare the effects of pH on the ability of amitriptyline to inhibit PEA deamination at normal and elevated oxygen tensions (Table 3). Unlike the iminodibenzyl analogues, amitriptyline inhibition of PEA deamination by human brain type B monoamine oxidase was similar at both pH 7.5 and 9.0. This pattern was observed at both normal and high oxygen concentrations.

DISCUSSION

The data presented in this paper demonstrate that a variety of tricyclic antidepressant and antipsychotic drugs inhibit human brain monoamine oxidase deami-

TABLE 2

Effect of pH on inhibition of phenylethylamine deamination by imipramine

See the legend to Fig. 4 for assay conditions.

pH	Inhibition	
	−Oxygen	+Oxygen
	%	%
7.0	38.2 ± 3.8	33.3 ± 7.9
7.5	49.0 ± 0.6	34.5 ± 8.7
8.0	57.4 ± 1.2	48.7 ± 3.3
8.5	58.6 ± 1.3	52.3 ± 1.9
9.0	57.2 ± 1.6	56.3 ± 3.1

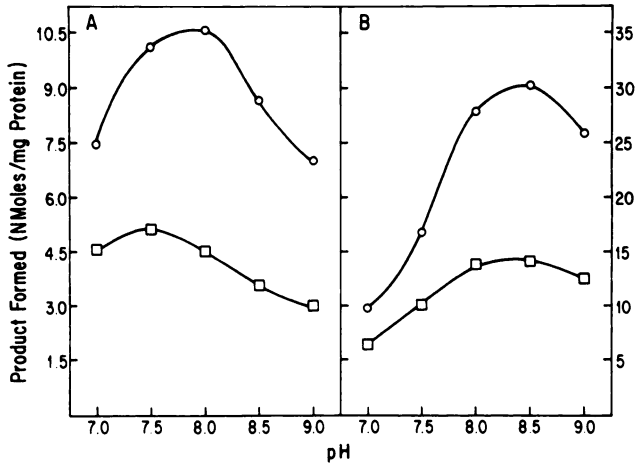

FIG. 4. *Effects of pH and oxygen on human brain monoamine oxidase deamination of 5.4 μM phenylethylamine in the absence* (○——○) *and presence* (□——□) *of 50 μM imipramine*

Reaction mixtures containing 0.27 mg of protein (normal oxygen tension, A) or 0.09 mg of protein (high oxygen tension, B) were incubated for 15 min at 37° in 2 ml of 0.04 M Tris-HCl buffer at the pH values indicated. Values shown are the averages of three experiments, each performed in triplicate.

TABLE 3

Percentage inhibition of phenylethylamine deamination by imipramine and desmethylimipramine at pH 7.5 and 9.0

Reaction mixtures containing 50 μM imipramine, desmethylimipramine, or amitriptyline, 0.27 mg of protein (low oxygen tension) or 0.09 mg of protein (high oxygen tension), and 5.4 μM [^{14}C]PEA were incubated for 15 min at 37° in 0.04 M Tris-HCl buffer. At normal oxygen tension and in the absence of drug, 2.82 ± 0.16 (SE) and 2.02 ± 0.03 nmoles of deaminated product were formed at pH 7.5 and 9.0, respectively; at high oxygen tension 1.56 ± 0.18 and 2.43 ± 0.07 nmoles of product were formed. Each value represents the mean ± standard error of three separate experiments, each performed in triplicate.

Drug	O_2	Inhibition		Ratio, pH 7.5:pH 9.0
		pH 7.5	pH 9.0	
		%	%	
Imipramine	−	47.3 ± 0.8	56.2 ± 1.2	0.81
Desmethylimipramine	−	42.9 ± 0.9	52.4 ± 0.7	0.78
Imipramine	+	36.0 ± 6.8	53.9 ± 1.6	0.50
Desmethylimipramine	+	30.6 ± 7.8	45.1 ± 3.9	0.53
Amitriptyline	−	68.4 ± 0.8	68.4 ± 1.1	1.00
Amitriptyline	+	71.4 ± 1.3	70.3 ± 1.6	1.02

nation of PEA. Of the clinically important drugs, amitriptyline was the most effective inhibitor of this reaction, followed in decreasing order of potency by chlorprothixene = nortriptyline > protriptyline > doxepin = imipramine = chlorpromazine > desmethylimipramine. Inhibition of PEA deamination ranged only from 40.8% to 66.9% for a 50 μM concentration of these drugs. In addition, the antidepressant agent iprindole was found to inhibit the human brain oxidase to approximately the same extent as did imipramine. Since iprindole does not inhibit biogenic amine reuptake in rat and mouse brain slices and in rat brain synaptosomal preparations (6–9), its ability to inhibit monoamine oxidase may be of some clinical relevance. Only the pharmacologically inactive analogues, chlorpromazine sulfoxide and imipramine *N*-oxide, failed to inhibit PEA deamination significantly. The inhibitor specificity of human brain type B monoamine oxidase for the tricyclic drugs is remarkably similar to that of the human platelet oxidase (10) and thus is consistent with other findings (12, 15) which suggest that human platelet monoamine oxidase is similar to the B form of the mitochondrial oxidase found in brain.

As pointed out in the INTRODUCTION, several laboratories have shown that the monoamine oxidase reaction proceeds via a ping-pong mechanism (13, 14). The first step in the reaction sequence involves deamination of the amine and reduction of the flavin cofactor; the second step consists of reoxidation of the flavin by oxygen. The over-all rate of this reaction, as indicated by the equation below, is dependent on both the concentration and Michaelis constant of the amine and oxygen (16).

$$v = \frac{V[RNH_2][O_2]}{K_{O_2}[RNH_2] + K_{RNH_2}[O_2] + [RNH_2][O_2]}$$

Accordingly, the rate-limiting step is controlled, in part, by the relative concentrations of the two substrates. Altering the concentration of either reactant would probably result in a change in the reaction sequence that is most rate-limiting. For example, in a previous article from this laboratory it was shown that the kinetics for inhibition of PEA deamination by amitriptyline was influenced by oxygen concentration (12). As oxygen was elevated, inhibition of the oxidase shifted from a mixed pattern to a more competitive one. These results were interpreted as suggesting that at high oxygen levels the deamination step becomes more rate-limiting and that the apparent competition for binding between amine substrate and amitriptyline, as reflected by Lineweaver-Burk plots, becomes more pronounced. It

was concluded that amitryptyline probably binds to both the oxidized and reduced forms of monoamine oxidase (12). Since a similar shift in the kinetics of inhibition of PEA deamination by imipramine, chlorpromazine, and chlorprothixene was observed in the present experiments, it may be concluded that these tricyclic drugs also inhibit both the oxidized and reduced forms of the enzyme.

The relative affinities and the degree to which these tricyclic drugs inhibit the oxidized and reduced forms of type B monoamine oxidase are apparently not equal, as evidenced by the different inhibition patterns observed in Lineweaver-Burk plots (Fig. 2) for these aromatic compounds. For example, chlorpromazine inhibited PEA deamination at normal oxygen levels almost uncompetitively, whereas at the same oxygen concentration imipramine and chlorprothixene displayed noncompetitive inhibition kinetics. Accordingly, it may be concluded that chlorpromazine inhibits the flavin reoxidation step to a greater extent than the flavin reduction step at normal oxygen tensions as compared with the other two drugs.

In addition to the effects of oxygen on tricyclic drug inhibition of monoamine oxidase, the data presented in this paper demonstrate that the interaction between the enzyme and these tricyclic drugs is also influenced by pH. As the pH of the incubation medium was increased, the percentage inhibitions of PEA deamination by imipramine and desmethylimipramine were likewise elevated. This is the opposite of what was observed when similar experiments were performed with rabbit brain monoamine oxidase (4). Although the increased inhibition of the human oxidase may suggest that the unionized species of these tricyclic compounds are principally responsible for inhibition of the oxidase, other experiments do not support this contention. Results in Table 3 reveal that the ratio of percentage inhibition between imipramine and desmethylimipramine remained constant at both pH 7.5 and 9.0. Thus, even though the concentration of the un-ionized form of imipramine at pH 9.0 was twice that of the desmethyl analogue, the relative ability of these compounds to inhibit PEA deamination remained the same at pH 7.5 and 9.0. In contrast to imipramine and desmethylimipramine, percentage inhibition of this reaction by amitriptyline was not affected by pH. This latter result further indicates that inhibition of monoamine oxidase is not regulated by the degree of ionization of tricyclic drugs.

The clinical relevance to tricyclic drug inhibition of biogenic amine deamination is of course unknown, although it has been reported that these drugs produce decreased levels of deaminated metabolites in brain (17–21), urine (22), and cerebrospinal fluid (23–25) of patients or experimental animals treated with these substances. These data, coupled to those presented here, suggest that tricyclic psychoactive drugs may inhibit monoamine oxidase *in vivo*. In fact, Sullivan *et al.* (26) have recently reported that platelet monoamine oxidase is decreased in depressed patients treated with amitriptyline or imipramine. Since these tricyclic drugs are also effective inhibitors of neuronal reuptake of amines, it is suggested that the two processes may act synergistically to promote the clinical efficacy of these important substances. However, further experimentation is needed to determine the relative contribution of monoamine oxidase inhibition by these aromatic drugs to their therapeutic action *in vivo*.

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