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Synthesis and Biological Activity of Two New Calcium-channel Blockers, Mebudipine and Dibudipine

M. MAHMOUDIAN, H. MIRKHANI, Z. NEHARDANI AND S. GHIAEE

Department of Pharmacology, Iran University of Medical Sciences, P.O. Box 14155-6183, Tehran, Iran

Abstract

Dihydropyridine derivative calcium-channel blockers are widely used in the therapy of hypertension, angina pectoris and other cardiovascular diseases. Because the prototype of dihydropyridine derivatives, nifedipine, does not have the optimum pharmacokinetic and pharmacodynamic characteristics, attempts have been made to synthesize other drugs in this class with improved properties. The synthesis and biological activity of two new calcium-channel blockers, non-symmetrical (mebudipine) and symmetrical (dibudipine) analogues of nifedipine, is described herein. The pharmacological potencies of the compounds were evaluated by studying their effects on the contractions of isolated guinea-pig ileum and rat aortic rings. Results were compared with those obtained from nifedipine.

The new analogues and nifedipine inhibited the contractile response of guinea-pig ileum to electrical stimulation and the pIC50 value of the compounds did not differ significantly from each other. The compounds also antagonized the contractile responses of K^+-depolarized guinea-pig ileum to cumulative concentrations of calcium. The inhibitory effect of mebudipine was significantly higher than that of nifedipine whereas the inhibitory effects of dibudipine and nifedipine were not different. All three compounds relaxed KCl (40 mM)–treated isolated aortic rings; the pIC50 values for relaxation were: mebudipine > nifedipine > dibudipine.

It is concluded that these new dihydropyridine derivatives are potent relaxants of vascular and ileal smooth muscles and therefore have high potential for use as antihypertensive and anti-anginal agents.

In the last two decades, calcium-channel blockers have found special significance in the therapy of hypertension, angina pectoris and other cardiovascular diseases (Weiner 1988). They can also be effective in non-cardiovascular medicine (Schwartz et al 1984). Among the classes of calcium-channel blockers, dihydropyridine derivatives are widely used because of their potent vasodilating activity and weak cardiodepressant action (Fleckenstein 1977). Because the prototype of dihydropyridines, nifedipine, does not have the optimum pharmacokinetic and pharmacodynamic characteristics, several attempts have been made to synthesize other drugs in this class with improved properties. The results of these efforts are new drugs with longer duration of action and fewer side effects. Some of these drugs have selectivity for the vessels and even for the vascular bed upon which they act (Freedman & Waters 1987).

In this study, two new analogues of nifedipine (Ghiaee & Mahmoudian 1995), mebudipine [(±)-*t*-butyl, methyl-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine dicarboxylate] and dibudipine [(±)-bis-*t*-butyl-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine dicarboxylate] (Fig. 1), have been synthesized and their biological activity as calcium-channel blockers in guinea-pig isolated ileum and rat isolated aorta has been examined. For comparison, nifedipine has been used as a standard compound.

Correspondence: M. Mahmoudian, Department of Pharmacology, Iran University of Medical Sciences, P.O. Box 14155-6183, Tehran, Iran.

Materials and Methods

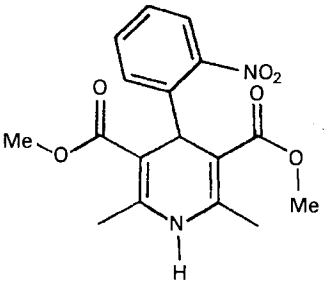
Drugs and solutions

Nifedipine was a gift from Tolid-darou Pharmaceuticals. All three calcium-channel blockers were dissolved in ethanol to furnish 1 mM solutions; these were then further diluted with distilled water. Solutions of dihydropyridines and the organ bath were protected from light. Other compounds were of analytical grade.

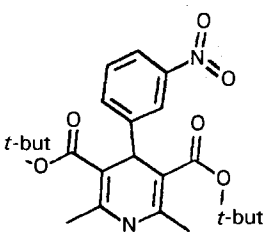
Synthesis of mebudipine ($C_{20}H_{24}N_2O_6$, MW 388·366)

The first step of the synthesis of mebudipine was the synthesis of *t*-butyl-2-(3-nitrobenzylidene) acetoacetate. A solution of 3-nitrobenzaldehyde (20·0 g, 132 mmol) and *t*-butyl acetoacetate (30·0 g, 190 mmol) was prepared in methanol (40·0 mL). Acetic acid was added to the methanolic solution at room temperature and after 10 min piperidine was added to the same solution. The solution was stirred for 4 h and the precipitate was collected by filtration and washed with water and light petroleum ether. Yellowish uniform crystals were obtained. The product was dried overnight in a vacuum desiccator to give 25·0 g (65%) pure product. The purity of the product was checked by thin-layer chromatography (TLC) as described below.

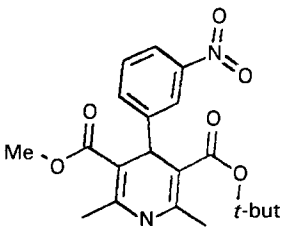
For synthesis of crude mebudipine *t*-butyl-2-(3-nitrobenzylidene) acetoacetate (25·0 g, 86 mmol) and methyl 3-aminocrotonate (12·5 g, 109 mmol) were dissolved in methanol (62·5 mL). The solution obtained was heated under reflux for 18 h (protected from light). The solution was cooled to 30°C and stirred at this temperature for 3 h, yielding a yellow precipitate which was collected by vacuum filtration and washed with ice-cold methanol (20·0 mL).



Nifedipine (MW = 346)



Dibudipine (MW = 430)



Mebudipine (MW = 388)

FIG. 1. The chemical structures of nifedipine, dibudipine and mebudipine.

For purification of mebudipine the crude product (26·5 g) was dissolved in hot (51–53°C) methanol (132·5 mL) with stirring. After cooling to 4°C, the precipitate was collected and dried in-vacuo (at 53–54°C) to give 16·5 g (49%) pure yellow product.

Synthesis of dibudipine ($C_{23}H_{30}N_2O_6$, MW 430·366)

For the synthesis of crude dibudipine, a solution of 3-nitrobenzaldehyde (20·0 g, 132 mmol) and *t*-butyl acetoacetate (60·0 g, 380 mmol) was prepared in methanol (40·0 mL). Ammonia solution (25%, 20·0 mL) was added to the methanolic solution and the mixture was heated under reflux for 10 h. The solution was cooled to 30°C and stirred at this temperature for 4 h, yielding a yellow precipitate which was collected by vacuum filtration and washed with ice-cold methanol (20·0 mL) to give a yellow product (24·0 g).

For purification of dibudipine crude dibudipine (10·0 g) was dissolved in methanol (70·0 mL) and the solution was heated under reflux for 30 min, cooled, and the precipitate was collected, washed with ice-cold methanol and dried in-vacuo to give the pure product (7·5 g, 32%).

TLC

Precoated TLC plates (silica gel 60, F_{254}, layer thickness 0·25 mm) with chloroform–methanol, 97·5:2·5, as mobile phase was used to monitor the syntheses and to check the purity of intermediates and finished products.

High-performance liquid chromatography (HPLC)

The purity and stability (rate of degradation) of mebudipine and dibudipine were determined by HPLC on a C_{18} column; water–acetonitrile–methanol, 50:25:25, was used as mobile phase at a flow rate of 1 mL min^{-1}.

Mass spectrometry

Low-resolution mass spectra were acquired with an MAT-CH5/DF (Finnigan) mass spectrometer; high-resolution mass spectra and accurate mass measurements were obtained with an A.E.I.-Kratos MS 30 spectrometer. Both spectrometers were coupled on line with a Data General DS 50 data system. Electron-impact ionization was performed at an ionizing energy of 70 eV; the source temperature was 250°C.

Nuclear magnetic resonance (NMR)

A 90 MHz FT NMR instrument (Jeol) was used to acquire NMR spectra; acetone-D_6 was used as solvent. The spectra obtained confirmed the structures of mebudipine and dibudipine.

Effects of mebudipine, dibudipine and nifedipine on ileum isolated guinea-pig

Male guinea-pigs, 200–400 g, were killed by a blow on the head after previously being deprived of food for 18 h but having had free access to water. The non-terminal part of the ileum was removed and cut into 20-mm long segments which were suspended in an organ bath and connected to an isotonic transducer. The organ bath contained 50 mL physiological solution oxygenated with 95% O_2 and 5% CO_2 at 37°C. The fluid of the organ bath was changed every 15 min. A resting tension of 0·5–1 g was applied to the ileal segments and they were left to equilibrate for 60 min.

To study the effects of mebudipine, dibudipine and nifedipine on electrically induced contraction of the ileum, the tissue was suspended in physiological solution (composition mM: NaCl 136·9, KCl 2·68, $MgCl_2$ 1·05, $CaCl_2$ 1·8, NaH_2PO_4 1, $NaHCO_3$ 11·9, glucose 5·5) and stimulated with a Harvard stimulator (stimulation specification: 25V, 0·1 Hz, pulse-width 5 ms) via a bipolar platinum electrode. Cumulative doses of each calcium antagonist were then added to the organ bath at 10-min intervals. Each segment was treated with only one compound. The pIC50 (−log IC50) value of each compound was calculated from concentration–response curves.

To study the effects of compounds on calcium-induced contractions, the tissue was suspended in a modified calcium-free, high potassium physiological solution of composition (mM): NaCl 97, KCl 40, NaH_2PO_4 0·4, $NaHCO_3$ 11·9, glucose 5·5. As a control, increasing concentrations of $CaCl_2$ (0·1, 0·3, 1, 3, 10 mmol) were added to an organ bath and the contractile response of the ileum was recorded. Tissues were then pre-incubated for 15–20 min with one concentration of each calcium antagonist and increasing concentrations of $CaCl_2$ were again added to the bathing media. Tissues were incubated with three concentrations of nifedipine and dibudipine and four concentrations of mebudipine. Each segment was treated with one compound only. To compare the inhibitory effects of mebudipine and dibudipine, the response percentage ratio (the ratio of the percent of maximum response induced by a particular concentration of calcium in the presence of a certain concentration of calcium-channel blocker and in its absence) was calculated and compared with that of nifedipine.

Effects of mebudipine, dibudipine and nifedipine on rat isolated aorta

White male rats were killed by a blow on the head and decapitation. The thoracic aorta was isolated, carefully dis-

sected from the surrounding tissues, and cut into 5 segments of 3 mm length. The segments were joined together, suspended in Krebs solution (composition mm: NaCl 118, KCl 4·8, $CaCl_2$ 2·5, KH_2PO_4 1·2, $MgSO_4$ 1·2, $NaHCO_3$ 25 and glucose 10) at 37°C and oxygenated with 95% O_2 and 5% CO_2. The fluid of the organ bath was changed every 15 min. The resting tension was adjusted to 1 g and the tissue was left to equilibrate for 1 h. The contractions of isolated aortic rings were recorded by means of a force transducer and Beckman physiograph.

To study the vasodilatory effects of mebudipine, dibudipine and nifedipine, the aorta was pre-contracted with KCl (40 mM) and increasing amounts of each calcium antagonist were then added to the organ bath. The pIC50 value of each compound was calculated from concentration-response curves.

Statistical methods

Results are expressed as means ± s.e. Differences between the pIC50 and response percentage ratio values of compounds in each preparation were compared by use of the two-tailed Student *t*-test. A P value $< 0{\cdot}05$ was considered to be indicative of significance.

Results

Chemistry

The structures of mebudipine, dibudipine and (for comparison) nifedipine are shown in Fig. 1. The scheme used for synthesis is presented in Fig. 2. The structures were confirmed by mass and NMR spectrometry, and results from HPLC analysis showed that the final products were pure. Both new compounds are readily soluble in acetone and chloroform, less soluble in ethanol and almost insoluble in water. Both are yellow crystalline powders. They were stable when exposed to daylight and to artificial light. After UV exposure, the formation of nitro- and nitrosophenyl-pyridine derivatives was lower than for nifedipine.

Effects on electrically induced contractions of the ileum

All three calcium-channel blockers concentration-dependently reduced electrically evoked contractile responses of guinea pig ileum. The concentration-response curves and pIC50 values are shown in Fig. 3 and Table 1, respectively. There were no significant differences between the results obtained with the two compounds. The highest percentage of ethanol which was obtained in organ bath was 0·1%; this had no significant effect on the electrically induced contractions (n = 4).

Effects on calcium-induced contractions of the ileum

Pre-treatment of the K^+-depolarized tissue with mebudipine, dibudipine and nifedipine resulted in a shift to the right of the calcium dose–response curves (Fig. 4). To compare the inhibitory effect of these compounds, the effect of 2×10^{-9} M of each compound on contractions induced by 3 mM calcium (which produces 84·44% (± 0·96) of maximum response in the absence of calcium antagonists) was considered. At this concentration, mebudipine, dibudipine and nifedipine reduced calcium (3 mM)–induced contractions to 29% (± 8), 80% (± 4) and 74% (± 6) of the maximum response, respectively. Under these conditions the response percentage ratio of mebudipine was significantly smaller than those of dibudipine ($P < 0{\cdot}001$) and nifedipine ($P < 0{\cdot}005$) whereas this parameter did not differ significantly for dibudipine and nifedipine (Table 2).

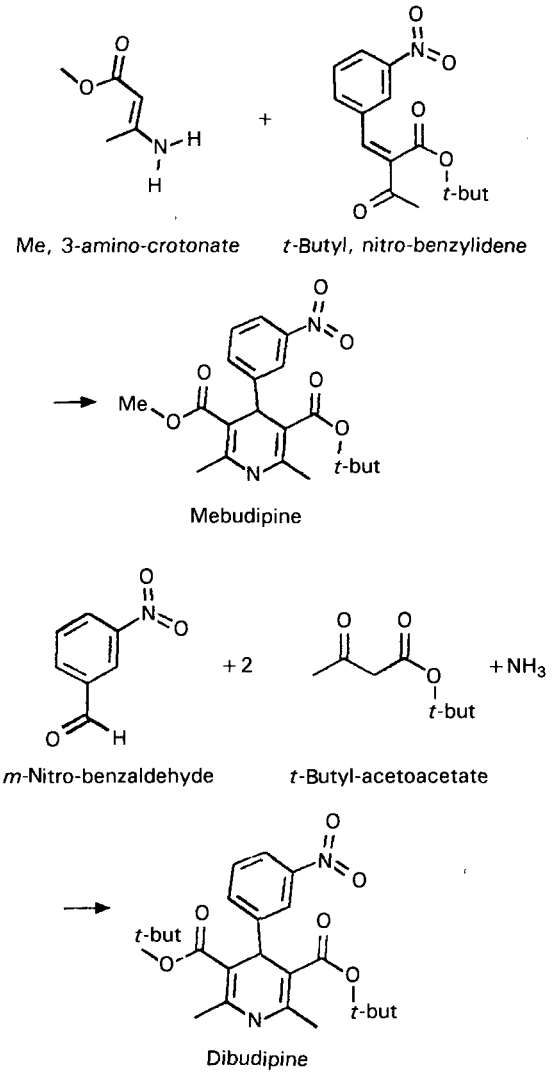


FIG. 2. Scheme for synthesis of mebudipine and dibudipine.

Therefore, the inhibitory effect of these compounds on calcium-induced contractions were mebudipine > dibudipine = nifedipine.

Effects on rat isolated aorta

Aortic rings pre-contracted with 40 mM KCl were relaxed by mebudipine, dibudipine and nifedipine (Fig. 5). The results indicated that the pIC50 of mebudipine was greater than that of nifedipine which in turn was greater than that of dibudipine. The differences among these values were statistically significant (mebudipine compared with nifedipine, $P < 0{\cdot}02$; nifedipine compared with dibudipine, $P < 0{\cdot}001$) (Table 1). Therefore, in rat isolated aorta, the potency of these drugs for relaxing K^+-induced contractions is mebudipine > nifedipine > dibudipine.

Discussion

Our results showed that symmetrical *t*-butyl and non-symmetrical methyl, *t*-butyl esters of dihydropyridines could easily be synthesized by one- and two-step reactions, respectively. The

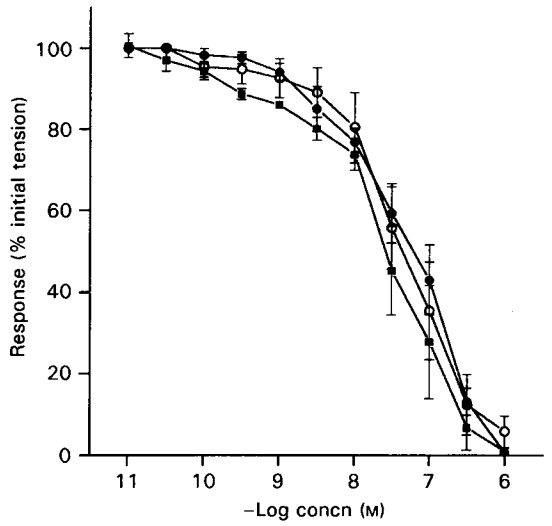


FIG. 3. The relaxing effect of increasing concentrations of mebudipine (■), dibudipine (●) and nifedipine (○) on the electrically induced contraction of guinea-pig isolated ileum (n = 5–7). Standard errors of the means are indicated by vertical lines.

Table 1. pIC50 (−log IC50) for relaxation by mebudipine, dibudipine and nifedipine of electrically induced contraction of guinea-pig isolated ileum and potassium-induced contraction of rat isolated aorta.

	Mebudipine	Dibudipine	Nifedipine
Ileum*	8·30 ± 0·32	7·58 ± 0·21	7·45 ± 0·27
Aorta**	8·61 ± 0·09	7·59 ± 0·12	8·29 ± 0·07

Values are means ± s.e. *In guinea-pig ileum there were no significant differences between pIC50 values. **In rat aorta, significance levels were: mebudipine compared with nifedipine $P < 0·02$, nifedipine compared with dibudipine $P < 0·001$.

final products were pure, stable compounds. Similar to other analogues of nifedipine, they are lipophilic compounds with slight solubility in water.

The results of biological study showed that these new dihydropyridines caused dose-dependent inhibition of the contractile responses induced by electrical stimulation (Fig. 3) and $CaCl_2$ (Fig. 4) in guinea-pig ileum. These effects were similar to those obtained with nifedipine, and could be explained as being caused by inhibition of entry of Ca^{2+} through voltage-dependent calcium channels in the muscle membrane (Schwartz 1989). In polarized tissues, all compounds inhibited to the same extent the contractions induced by electrical stimulation and their pIC50 values did not differ significantly from each other (Table 1). Results from K^+-depolarized tissues (Table 2) demonstrated that the inhibitory effect of mebudipine on responses to Ca^{2+} was greater than that of nifedipine. However, the effect of dibudipine was statistically similar to that of nifedipine. Surprisingly, in the presence of different concentrations of each calcium-channel blocker, the maximum responses of ileal segments were attained at similar concentrations of calcium (i.e. 10 mM; Fig. 4). Similar results have been reported by investigators who studied the effect of calcium-channel blockers on guinea-pig isolated atria (Zonta et al 1992). It has been reported that dihydropyridines are more effective in depolarized tissue

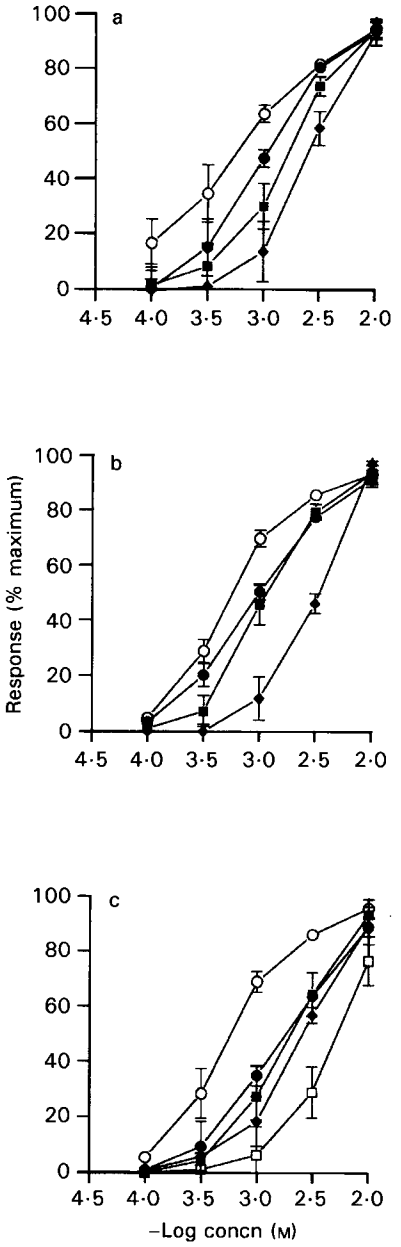


FIG. 4. The inhibitory effect of nifedipine (a), dibudipine (b) and mebudipine (c), on the calcium-induced contraction of guinea-pig isolated ileum. In plots a and b, ○ represents the control, ● the effect of 2×10^{-10} M, ■ the effect of 2×10^{-9} M and ● the effect of 2×10^{-8} M nifedipine or dibudipine on calcium-induced contraction. In plot c, ○ represents the control, ● the effect of 2×10^{-12} M, ■ the effect of 2×10^{-11} M, ● the effect of 2×10^{-10} M and □ the effect of 2×10^{-9}M mebudipine on calcium-induced contraction. Responses are shown as a percentage of the maximum response in the absence of calcium-channel blocker. Standard errors of the means are indicated by vertical lines (n = 5 or 6).

(Morel & Godfraind 1994). Although the potency of mebudipine in relaxing electrically induced contractions of isolated ileum (polarized tissue) was equal to those of dibudipine and nifedipine (Table 1), its inhibitory effect on calcium-induced contractions (in K^+-depolarized tissue) was significantly greater than those of the other two compounds (Table 2), so it seems that mebudipine is more selective than the other drugs toward depolarized tissue. However, it should be taken into

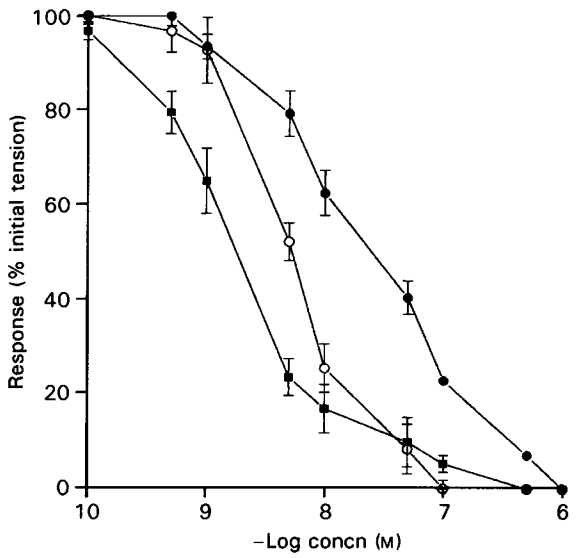


FIG. 5. The relaxing effect of increasing concentrations of mebudipine (■), dibudipine (●) and nifedipine (○) on the potassium-induced contraction of isolated rat aorta (n = 6–8). Standard errors of the means are indicated by vertical lines.

account that a complex network of neuronal and muscular tissues exists in the intestine and we could not rule out the possibility of different action of the above compounds at several neuronal and muscular sites on which they might act.

The study of the effect of mebudipine and dibudipine on rat isolated aorta has shown that they are potent vasodilators (especially mebudipine). These results indicate that the vasorelaxant effect of mebudipine is greater than that of nifedipine. However, nifedipine is more potent than dibudipine in this regard (Table 1).

It has been reported that asymmetric esters of dihydropyridines are generally more potent than symmetric esters (Ohtsuka et al 1989). The results of the current study are in agreement with this.

In conclusion, this preliminary study shows that mebudipine and dibudipine are potent relaxants of vascular and ileal smooth muscles. Therefore, they have high potential for use in disorders (e.g. hypertension and angina pectoris) normally treated with calcium-channel blockers.

Table 2. Response percentage ratio of mebudipine, dibudipine and nifedipine on calcium-induced contraction in guinea-pig ileum.

	Mebudipine	Dibudipine	Nifedipine
Response percentage ratio	0·35 ± 0·10	0·92 ± 0·04	0·90 ± 0·07

Response percentage ratios were calculated by dividing the percent of maximum responses induced by 3 mM calcium in the presence of calcium-channel blocker (2×10^{-9} M) by the percent of maximum response of the same concentration of calcium in the absence of the calcium antagonist. The response percentage ratio of mebudipine differs significantly from those of dibudipine ($P < 0.001$) and nifedipine ($P < 0.005$).

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