Interaction between isoniazid and diverse vasodilators: role of decreased cerebral GABA

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Abstract

Objective: To determine if the interaction between isoniazid and hydralazine, consisting of increased hypotension accompanied by bradycardia, occurs with other vasodilators. Methods: Blood pressure and heart rate responses to a number of vasodilators were determined in rats under chloralose-urethane, pretreated or not with 250 mg/kg of isoniazid. The influence of this dose of isoniazid on GABA levels in the hypothalamus and pons-medulla was assessed in other groups of rats. Results: Increased hypotension and bradycardia following i.p. isoniazid were observed with dipyridamole, prazosin, pinacidil and hydralazine given i.v. Bradycardia without increased hypotension appeared with papaverine and verapamil, while increased hypotension with unchanged heart rate was observed with minoxidil and captopril. Isoniazid decreased GABA in the hypothalamus and pons-medulla. Conclusions: At the high dose used, isoniazid interacts with various vasodilators, irrespective of their mechanism of action. The interaction could be due to the influence of the drug on GABA levels at cardiovascular regulatory sites. © 1998 Elsevier Science B.V.

Keywords: Isoniazid; Vasodilator; Hypotension; Bradycardia; Rat

1. Introduction

It has been previously reported that in the rat, a number of hydrazine derivatives potentiate the hypotensive effects of the vasodilator hydralazine (HYD) and transform the accompanying reflex tachycardia to bradycardia [1]. The interaction appeared to be specific for HYD, since it could not be reproduced with the α-adrenergic antagonist prazosin (PRA).

A well-known effect of hydrazine derivatives is to decrease the synthesis of the neurotransmitter γ-amino-butyric acid (GABA) through reduction in the availability of pyridoxal necessary for the activity of glutamic acid dehydrogenase, a crucial enzyme in the synthesis of GABA [2]. Although GABA inhibition could be involved in the above interaction, such possibility could not be substantiated in that study, mainly because all hydrazines were tested at 10 mg/kg, a dose at which only some of them could be expected to affect cerebral levels of the amino acid.

In this context, the case of isoniazid (ISO), one of the most potent hydrazines detected, seemed particularly interesting, since its GABA-depleting effect occurs at 250 mg/kg [3,4], a dose 25 times greater than that used in the above study. It therefore seemed of interest to determine whether a dose of ISO high enough to reduce cerebral GABA is also capable of interacting with HYD and whether at this dose, the interaction is specific for HYD, as appeared to be the case in the previous study, or extends to other vasodilator agents. In the present work, these issues were investigated and the GABA-reducing effect of ISO under the existing experimental conditions was verified by measuring amino acid levels in the hypothalamus and pons-medulla of control and ISO-pretreated animals. The

Abbreviations: CAP, captopril; DIP, dipyridamole; GABA, γ-amino-butyric acid; HYD, hydralazine; ISO, isoniazid; MIN, minoxidil; 3MP, 3-mercaptopropionic acid; PAP, papaverine; PIN, pinacidil; PRA, prazosin; VER, verapamil

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experiments were carried out in rats anesthetized with chloralose-urethane, in order to avoid the enhancement of GABAergic transmission produced by other anesthetics such as the barbiturates [5], which could theoretically mask the expected interaction between ISO and the test cardiovascular agents.

2. Materials and methods

2.1. Blood pressure experiments

Male Wistar rats weighing between 200 and 360 g were anesthetized with a mixture of chloralose, 40 mg/kg, and urethane, 1200 mg/kg, administered i.p. Cannulas were placed in the trachea, a femoral artery and a femoral vein for artificial ventilation, blood pressure recording and drug administration, respectively. Blood pressure was recorded on a Model 79 Grass polygraph with a Statham-Gould P23ID transducer, the output of which was electronically dampened to obtain mean pressure. The undampened signal from the transducer was used to drive a Grass 7P4 cardiotachograph for recording of heart rate on another channel of the polygraph. In all experiments, groups of 10 animals were used, recordings were made for 120 min after drug administration and changes in the parameters studied were tabulated at 10-min intervals.

Blood pressure and heart rate responses to various vasodilators were obtained in either unpretreated control animals or in rats pretreated 30 min previously with ISO, 250 mg/kg i.p. The vasodilators used and their test doses in mg/kg were papaverine (PAP) 10, verapamil (VER) 0.5, captopril (CAP) 0.2, minoxidil (MIN) 1, dipyridamole (DIP) 10, PRA 0.005, pinacidil (PIN) 0.2 and HYD 0.1. These doses were chosen to reduce blood pressure in control rats by 10 to 25 mmHg, 10 min after injection. As an additional control, another pair of unpretreated and ISO-pretreated groups were injected with isotonic saline solution, 1 ml/kg, instead of the vasodilators.

In order to more clearly determine whether the influence of ISO pretreatment on responses to the vasodilators could be attributed to an interaction with the direct vascular effect of the test drugs or to interference with homeostatic adjustments in response to the acute hypotension produced, an additional set of control and ISO-pretreated rats received an infusion of PRA, 0.1 μg/kg per min, over 60 min. Recordings were continued for another 60 min after stopping the infusion.

Finally, the possible influence of ISO on baroreflex function was assessed in experiments in control and pretreated rats receiving bolus injections of phenylephrine, 10 μg/kg. In each animal, blood pressure increases and heart rate decreases after three such injections were averaged and the resulting means were taken as indicators of baroreflex sensitivity.

The procedures followed conform with the ‘Guide for the Care and Use of Laboratory Animals’ published by the U.S. National Institutes of Health (NIH publication No. 85-23, revised 1985).

2.2. Determination of cerebral GABA

In these experiments, endogenous levels of glutamate, aspartate, taurine and glycine were measured in conjunction with GABA, considering that these amino acids also subserve neurotransmitter roles in the nucleus tractus solitarii [6] and could therefore be involved in cardiovascular regulation. Rats were anesthetized as described for the blood pressure experiments and received ISO, 250 mg/kg i.p. or were left unpretreated. Thirty min later, all rats were injected with the GABA synthesis inhibitor 3-mercapto-propionic acid (3MP), 65 mg/kg i.v., in order to prevent the postmortem increase in GABA [7] and were decapitated after 2 min. The brain was removed and the pons-medulla and hypothalamus were dissected, weighed, homogenized in methanol, centrifuged and subjected to analysis. Successful sample processing was achieved in 11 pons-medulla and in 12 hypothalamus preparations. The amino acids were determined by precolumn o-phthaldialdehyde derivatization and reversed-phase high-performance liquid chromatography with fluorescence detection [8].

2.3. Statistical procedures

Statistical analysis of the results was carried out using an Instat2 software package. Since the non-homogeneous variances detected in some of the experimental groups precluded the use of parametric procedures, non-parametric tests were applied. Homogeneity among baseline blood pressure and heart rate values of the 22 experimental groups was determined by the Kruskal–Wallis procedure and the corresponding control and ISO groups were subsequently compared by Dunn’s test for selected pairs. Pressure and rate changes in the control and ISO groups at different times were compared by the Mann–Whitney test. In all cases, a probability value less than 0.05 was taken as indicative of statistical significance of the differences observed.

2.4. Drugs

The hydrochlorides of PAP, (±)-VER, PRA, HYD and L-phenylephrine, as well as the free bases or acids of MIN, CAP, ISO and 3MP were obtained from Sigma Chemical, St. Louis, MO. DIP and PIN free bases were purchased from Research Biochemicals Inc., Natick, MA. Doses refer to those forms of the drugs. DIP and PIN were dissolved with the aid of a drop of concentrated hydrochloric acid; PRA was dissolved in 3% ethanol. All other drugs were dissolved in isotonic saline solution. The volume of drug solution administered was 2 ml/kg for ISO and 1 ml/kg.
Table 1
Baseline blood pressure and heart rate values and immediate responses in the different experimental groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean blood pressure (mmHg)</th>
<th>Heart rate (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Isoniazid</td>
</tr>
<tr>
<td>Saline</td>
<td>128 ± 4</td>
<td>110 ± 6</td>
</tr>
<tr>
<td>Papaverine</td>
<td>124 ± 4</td>
<td>119 ± 4</td>
</tr>
<tr>
<td>Verapamil</td>
<td>123 ± 5</td>
<td>109 ± 4</td>
</tr>
<tr>
<td>Captopril</td>
<td>124 ± 4</td>
<td>117 ± 4</td>
</tr>
<tr>
<td>Minoxidil</td>
<td>116 ± 4</td>
<td>126 ± 4</td>
</tr>
<tr>
<td>Dipyramidol</td>
<td>120 ± 3</td>
<td>108 ± 4</td>
</tr>
<tr>
<td>Prazosin bol</td>
<td>122 ± 5</td>
<td>107 ± 4</td>
</tr>
<tr>
<td>Prazosin inf</td>
<td>121 ± 3</td>
<td>128 ± 4</td>
</tr>
<tr>
<td>Pinacidil</td>
<td>126 ± 3</td>
<td>124 ± 4</td>
</tr>
<tr>
<td>Hydralazine</td>
<td>127 ± 4</td>
<td>114 ± 5</td>
</tr>
<tr>
<td>Phenytoinephrine</td>
<td>118 ± 5</td>
<td>120 ± 5</td>
</tr>
</tbody>
</table>

All values are means ± S.E.M., n = 10; Δ = change in blood pressure or heart rate immediately after drug; bol = bolus; inf = infusion; *P < 0.05 vs. corresponding control.

for all other agents; in the infusion experiments with PRA, the volume delivered was 0.025 ml/min.

3. Results

Baseline blood pressure and heart rate values in the different experimental groups are shown in Table 1. It is apparent that pressures in the control and ISO groups varied appreciably, i.e., between 116 and 128 mmHg in controls and between 107 and 128 mmHg in pretreated rats. Statistical analysis of the data revealed that values for the 22 groups were indeed significantly non-homogenous. However, such variation could not be attributed to ISO pretreatment, since no significant differences were found.

Fig. 1. Blood pressure (upper panels) and heart rate (lower panels) responses to saline, verapamil and papaverine in control (○) and isoniazid-pretreated (□) rats. Symbols represent means of 10 animals; vertical lines are S.E.M. Asterisks denote significant differences from control. Abscissae correspond to time after drug injection; ordinates, to differences in mmHg or beats/min from pre-injection values.
Fig. 2. Blood pressure (upper panels) and heart rate (lower panels) responses to captopril, minoxidil and dipyridamole in control (●) and isoniazid-pretreated (○) rats. Symbols represent means of 10 animals; vertical lines are S.E.M. Asterisks denote significant differences from control. Abscissae correspond to time after drug injection; ordinates, to differences in mmHg or beats/min from pre-injection values.

Fig. 3. Blood pressure (upper panels) and heart rate (lower panels) responses to pinacidil, prazosin and hydralazine in control (●) and isoniazid-pretreated (○) rats. Symbols represent means of 10 animals; vertical lines are S.E.M. Asterisks denote significant differences from control. Abscissae correspond to time after drug injection; ordinates, to differences in mmHg or beats/min from pre-injection values.
Fig. 4. Mean blood pressure (left panel) and heart rate (right panel) responses to saline and vasodilators in control and isoniazid-pretreated rats. Bars represent differences in mmHg and beats/min with pre-vasodilator values over 120 min of observation. Vertical lines denote S.E.M. and asterisks, significant differences between isoniazid-pretreated groups and their corresponding controls.

when corresponding control and ISO groups were compared. Baseline heart rates were not significantly different among groups.

Figs. 1–3 show pressure and rate changes after saline and the vasodilators tested. In control rats receiving saline, blood pressure tended to increase over the 120 min of observation, while in those treated with the vasodilators it decreased but for not more than 60 min after administration. MIN was an exception, since hypotension slowly increased in magnitude during the experiments. In ISO-pretreated animals, the pattern of pressure changes after saline did not differ from that of the non-pretreated group;

Fig. 5. (A) Blood pressure (upper panel) and heart rate (lower panel) responses to an i.v. infusion of prazosin, 0.1 µg/kg per min for 60 min in control (●) and isoniazid-pretreated (○) rats. Symbols represent means of 10 animals; vertical lines are S.E.M. Asterisks denote significant differences from controls. Horizontal bars indicate the period of infusion. (B) Results of experiments in A expressed by averaging pressure and rate responses to prazosin infusion over 30-min periods. The first and second pairs of bars represent changes over minutes 10 through 30 and 40 through 60 of infusion; the third and fourth pairs are changes over minutes 70 through 90 and 100 through 120 of recovery. Vertical lines denote S.E.M. and asterisks, significant differences between isoniazid and control.
the vasodilators induced greater falls in blood pressure, potentiation being specially prominent with MIN, DIP, PIN, PRA and HYD. Heart rate of control rats tended to increase over time after administration of either saline or the vasodilators, this trend being preceded by slight bradycardia after VER, PAP, CAP, DIP and HYD. After ISO pretreatment, the increase in rate was prevented in the saline and VER groups, was unchanged with CAP and MIN and was reversed to bradycardia in the other groups.

In order to have a more quantitative estimate of the influence of ISO on responses of anesthetized rats to the vasodilators, the mean of the overall changes in pressure and rate during the 120 min of observation was calculated for all groups of control and ISO rats. Values for each pair of groups were compared statistically and are shown in Fig. 4. It is apparent that ISO pretreatment did not modify the changes observed after saline. Pretreatment did not influence pressure responses to PAP and VER, but it did favor the appearance of bradycardia after these drugs. The opposite pattern, i.e., increased hypotension and unchanged rate response, was observed with CAP and MIN. The rest of the vasodilators showed both potentiated pressure falls and bradycardia.

In control rats, all drugs except MIN and HYD elicited an immediate fall in pressure with a maximum occurring at less than 10 min and therefore not shown in Figs. 1–3. These decreases were accompanied by bradycardia with all agents except PIN, in which tachycardia was observed. Pretreatment with ISO did not affect these responses (Table 1). The results of experiments with infusions of PRA to further explore this issue are shown in Fig. 5. The hypotension elicited by PRA in control rats was not sustained during infusion and disappeared within 60 min after its discontinuation. ISO pretreatment enhanced this response both during and after infusion and prevented complete recovery. Heart rate did not change throughout infusion in either group and increased somewhat after discontinuation in control, but not in pretreated rats.

As shown in Table 1, phenylephrine produced the expected rise in blood pressure and reflexly decreased heart rate. The pressor response was unchanged by ISO pretreatment, but the accompanying bradycardia was significantly reduced.

The effect of ISO on regional brain levels of amino acids is shown in Table 2. The only significant changes observed were decreases in GABA concentrations in both pons-medulla and hypothalamus, amounting to 35 and 42%, respectively.

4. Discussion

The present results show that, when administered at a dose high enough to decrease cerebral levels of GABA, ISO is capable of interacting with other vasodilators besides HYD, and that this phenomenon results in an increased and more prolonged hypotensive response accompanied by persistent bradycardia, as reported earlier [1]. This finding suggests that the interaction is independent of the mechanism of action of the vasodilator involved, since it occurs with agents eliciting phosphodiesterase inhibition (PAP), calcium channel blockade (VER), angiotensin converting enzyme inhibition (CAP), potassium channel opening (MIN and PIN), enhancement of adenosine action (DIP), α-adrenergic blockade (PRA) or vascular smooth muscle relaxation by other as yet unknown mechanisms (HYD).

In some cases the interaction was incomplete, since it lacked either the potentiated hypotension or the bradycardic components. The former pattern was apparent with PAP and VER. The vascular smooth muscle relaxation induced by PAP, while related to phosphodiesterase inhibition [9], shows the pattern characteristic of calcium antagonists [10], so that in a sense this drug resembles VER. It would therefore appear that lowering of blood pressure through calcium channel blockade is not amenable to potentiation by ISO. Increased hypotension without bradycardia was observed with MIN and CAP. The lack of bradycardia after MIN underlines the importance of immediate pressure and/or rate changes for the interaction to take place. Maximum responses to this drug appear between 4 and 6 h after administration [11] to allow generation of an active metabolite [12]. In the case of CAP, the absence of bradycardia could be related to the fact that this agent facilitates vagal cardiac slowing [13], thus precluding further facilitation by ISO (see below).

Explanation of the increased hypotensive response to vasodilators in terms of ISO-induced decreased levels of GABA does not seem straightforward. Central administration of the amino acid, as well as of other GABA receptor agonists, produces hypotension, while the opposite effect is observed with the corresponding antagonists [14] or with GABA synthesis inhibitors [15,16]. Thus, direct involvement of reduced GABA levels in the hypotensive component of the ISO–vasodilator interaction appears untenable. It should be noted that microinjections of GABA agonists or antagonists in certain areas, notably the nucleus tractus solitarii [17] and the caudal ventrolateral medulla [18].
produce pressure and rate effects opposite to the above. In view of the generalized ISO-induced reduction of GABA levels throughout the brain [19], it is difficult to visualize a localized effect at these sites after systemic administration of the drug.

A different type of involvement of GABA in blood pressure regulation is suggested by the observation that drug-induced hypotension is accompanied by decreased GABA release in the rostral and caudal ventrolateral medulla [20,21], the locus coeruleus [22] and the paraventricular nucleus [23]. It is hypothesized that the decreased GABA production is a compensatory reaction leading to reduced inhibition of structures regulating blood pressure and thereby to accelerated recovery from the hypotension [24]. Interference by ISO with this reaction in the present experiments would intensify vasodilator-induced hypotension.

The immediate pressure fall produced by some of the vasodilators (Table 1) can be considered to represent exclusively the relaxant effect of these agents on vascular smooth muscle, whereas the gradual disappearance of such response is due not only to drug removal from active sites, but also to homeostatic mechanisms opposing the sudden hypotension produced. The failure of ISO to modify this immediate hypotension (Table 1) suggests that the drug does not influence vascular smooth muscle relaxation, but rather interferes with central mechanisms involved in recovery from acute hypotension. An interaction of a peripheral type has been observed in vitro with ISO and nitroglycerin and has been attributed to an increased availability of sulphydryl donors for bioactivation of the nitrovasodilator [25]. It is obvious that such a mechanism would not apply to the present results.

The issue of the influence of ISO on late as opposed to immediate vasodilator-induced hypotension was further explored by administering PRA by infusion, rather than by a single bolus. It was reasoned that hypotension observed early in the infusion period could represent mainly the direct vascular effect of the drug, while that occurring later on, would be the resultant of homeostatic mechanisms interacting with this direct effect. The failure of PRA to elicit sustained hypotension during infusion in control rats supports this possibility. Although ISO pretreatment enhanced the depressor response throughout the experiments, potentiation tended to be somewhat greater in the last 30 min of infusion and specially during recovery, suggesting a greater influence of the drug on the homeostatic component of the depressor response. It is possible that at the infusion rate of PRA chosen, complete separation of direct and indirect influences on blood pressure was not achieved, the latter being already present at the onset of hypotension.

The bradycardia observed after the vasodilators in ISO-pretreated rats could be due to facilitation of the decrease in rate produced by the drugs, either normally or under certain experimental conditions. Decreased heart rate would be expected with agents interfering with calcium inflow into myocardial cells either directly (VER) or indirectly (PIN) [26]. Bradycardia due to a variety of mechanisms different from their vascular effects has also been observed with some of the other vasodilators. Thus, the negative chronotropic effects of PAP have been attributed to local anesthesia [27], those of DIP to a vagal reflex of the Bezold-Jarisch type [28] or to increased myocardial adenosine levels [29], those of PRA to vagal stimulation [30] and those of HYD to cardiac muscarinic receptor activation [31]. Potentiation of this response could be produced by ISO through interference with the inhibitory influence of GABA on cardiac parasympathetic tone. Indeed, injection of ISO in the nucleus ambiguus, site of the cell bodies of the vagal projection to the heart, produces bradycardia, an effect attributed to inhibition of GABA synthesis [32]. In addition, since, as shown in the phenylephrine experiments, ISO reduced the sensitivity of the baroreflex, the positive chronotropic component of this reflex, normally evoked by vasodilator-induced hypotension, would be blunted after ISO and the negative chronotropic effects of the test agents allowed to above would become more prominent. Reduction of baroreflex sensitivity by ISO can also be attributed to central GABA depletion, since similar effects have been described with the GABA synthesis inhibitor 3MP [33] and the GABA receptor antagonist picrotoxin [34].

The present results illustrate the importance of GABAergic influences on responses to vasodilators. Their practical applicability is limited by the fact that such influences are observed with a dose of ISO similar to its convulsant ED50 in rats of 247 mg/kg [35]. The possibility of eliciting the interaction by concurrent repeated administration of vasodilators and lower, better tolerated doses of ISO within the range of those used clinically (5–10 mg/kg), was not explored in the present study, thus limiting the physiological implications of the reported findings. It should be mentioned, however, that after chronic administration of such doses, the effect of ISO on cerebral GABA is reversed and increased, rather than decreased levels of the amino acid are observed [36]. In fact, treatment of patients with Huntington’s chorea for 6 weeks with tolerated doses of ISO produces a 3-fold increase in cerebrospinal fluid concentration of GABA [37]. Such paradoxical effects are the result of preferential inhibition at these doses of GABA transaminase, another pyridoxal-dependent enzyme involved in the breakdown of the neurotransmitter.

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