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Pretreatment Effects on Nitrite-Induced Methemoglobinemia: Saline and Calcium Channel Antagonists

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FAHEY, J. M. AND R. L. ISAACSON. *Pretreatment effects on nitrite-induced methemoglobinemia: Saline and calcium channel antagonists.* PHARMACOL BIOCHEM BEHAV **37**(3) 457–459, 1990.—This study was undertaken to evaluate the effects of pretreatment with calcium channel antagonists (nimodipine or verapamil) on the formation of methemoglobin produced by sodium nitrite. Unexpectedly, the pretreatment of animals with control injections of physiological saline 2 hours before the nitrite administration reduced the amount of methemoglobin found in the blood 25 minutes later. When either of the calcium channel antagonists was given 2 hours before the administration of sodium nitrite, the saline effect was eliminated. When the injections of physiological saline or either of the calcium channel blockers were divided 24 hours before the nitrite administration, all reduced the amount of methemoglobin formed relative to rats that received no pretreatment. A tentative hypothesis is that the reduction of the nitrite-induced methemoglobin can be induced by the stress of handling and intraperitoneal injection and that this stress effect can last at least 24 hours. It is likely that whatever stress-related mechanism is involved in reducing methemoglobin levels, this effect can be reduced by the presence of "L channel" voltage-sensitive calcium antagonists or their active metabolites.

Nimodipine Verapamil Methemoglobin Sodium nitrite Calcium

SODIUM nitrite ($NaNO_2$) produces cerebral hypoxia through a dual pathway (16). The nitrite ion in $NaNO_2$ directly oxidizes hemoglobin (Hb) to methemoglobin (MHb) and consequently reduces the amount of oxygen available for respiration (17). The formation of MHb also impairs the release of oxygen from unaffected Hb as well, producing an even greater degree of hypoxia than would be anticipated on the basis of the percentage of MHb measured (3). In addition, $NaNO_2$ produces a hypotensive state as a result of the relaxation of smooth muscle. This also could act to exaggerate the level of hypoxia induced by a particular dose of the nitrite.

Under physiologic conditions, MHb is continuously being formed within erythrocytes by the oxidation of Hb, but it is prevented from accumulating within the red blood cell by a specific NADH diaphorase system called MHb reductase. Larger amounts of MHb are pathogenic and are found in the blood when the rate of formation and the amount of MHb overwhelm the reducing enzyme system.

This study was undertaken to investigate the effect of nimodipine and verapamil on $NaNO_2$-induced methemoglobinemia. Since there is a striking similarity of changes in brain anatomy (10) produced by the systemic administration of sodium nitrite to those associated with hypoxia produced by classical means, and, furthermore, the protective effect of calcium L channel antagonists, like nimodipine and verapamil, on hypoxia and ischemia is well documented (9,14), it might be expected that these drugs would be helpful in the reduction of $NaNO_2$-induced hypoxic deficits. Along this same line, nimodipine and other L channel antagonists can exaggerate the cardiovascular and behavioral changes produced by depressant drugs [e.g., (2, 9, 12, 13)] that, themselves, tend to reduce ischemic damage. On the other hand, $NaNO_2$ has known vasodilation effects that may be related to the amount of MHb produced by a particular amount of the drug (4). The L channel antagonists exert a similar effect since they are also potent vasodilators (7). Therefore, an unambiguous hypothesis about the interaction of L channel antagonists and sodium nitrite in regard to MHb formation cannot be formed and an empirical approach must be taken.

METHOD

One hundred and twelve nonselectively bred, experimentally naive, male Long-Evans hooded rats, 45–55 days of age from a large colony maintained at SUNY Binghamton, were used in this study. They were housed in single cages in a vivarium ($20 \pm 2°C$) on a 12:12 hour dark:light schedule with food and water available ad lib. The procedures were carried out during the light period. All drugs were dissolved in 0.09% physiological saline (PBS) and

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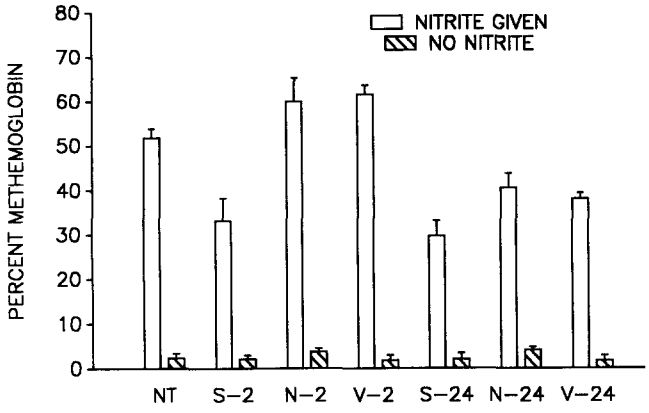

FIG. 1. The percent methemoglobin found in the blood of animals 2 or 24 hours after saline or calcium channel blocker administration and 25 min after sodium nitrite administration or no such injection. The different groups in the study are represented along the x-axis. Group NT received no injection prior to the sodium nitrite and blood samples were taken 25 min later. The groups designated with the letters S, N, and V indicate groups pretreated with saline, nimodipine, or verapamil, respectively. The numbers following the letters indicate the number of hours the saline or the drugs were given before the time of nitrite administration, i.e., 2 indicates a 2-hr interval; 24 indicates a 24-hr interval.

were administered intraperitoneally (IP) in an injected volume of 1.0 ml/kg.

The rats were then randomly divided into four groups based on the type of pretreatment that they would receive: nimodipine (5.0 mg/kg), verapamil (5.45 mg/kg), equivalent volumes of PBS (vehicle controls), or no treatment at all (treatment controls). Animals in the first three groups were then subdivided into two categories depending on whether the injections were given 2 or 24 hours before blood sampling. Finally, one-half of the animals in all groups were given $NaNO_2$ (75.0 mg/kg) 25 minutes prior to testing. The samples of blood needed for the determination were obtained by cardiac puncture (15) subsequent to ether-induced hypnosis. Methemoglobin levels were calculated as a percentage of total Hb using a spectrofluorometric technique (6). In all, 14 groups of rats were tested with 8 animals in each group.

RESULTS

The mean ($\pm$SEM) MHb levels for all groups of rats are presented in Fig. 1. The administration of $NaNO_2$ enhanced the amount of MHb in the blood beyond the low levels found in the animals receiving only the PBS vehicle. There was no effect on MHb levels of the administration of PBS, rather than nitrite, 25 min before testing. A one-way ANOVA applied to the data from the $NaNO_2$ treatment groups indicated a significant overall effect, $F(6,55) = 12.97$, $p<0.001$. Post hoc Scheffe comparisons indicated that the MHb level of the group that received no injection before the nitrite administration was higher than that of both $NaNO_2$-treated saline groups, that is, both those receiving saline injections 2 and 24 hr before the nitrite administration ($p\leq0.01$ and $p\leq0.05$, respectively). The group with no treatment prior to the nitrite did not differ from the groups of rats pretreated with nimodipine or verapamil 2 hours before $NaNO_2$, but its level was higher than the groups pretreated with these drugs 24 hours before $NaNO_2$ administration ($ps\leq0.05$). The two groups given the nimodipine or verapamil 2 hours prior to $NaNO_2$ had higher MHb levels than either of the $NaNO_2$-treated PBS groups ($p\leq0.01$). There was no difference found in the amount of MHb formed by $NaNO_2$ between the two calcium antagonist groups at either pretreatment time. In the 24-hour pretreatment condition, neither of these groups differed from the PBS-pretreated rats given $NaNO_2$.

DISCUSSION

As anticipated, the administration of $NaNO_2$ substantially increased MHb levels in all groups to which it was given, and there was no effect of the calcium antagonists or saline, by themselves, on MHb levels. However, an unanticipated effect was observed. Pretreatment with PBS either 2 or 24 hours prior to $NaNO_2$ administration reduced MHb levels relative to animals that did not receive any pretreatment. These results reveal an unusual effect of a PBS injection, as well as the associated handling, on methemoglobinemia induced at a later time by sodium nitrite. The stress of handling and PBS administration is most likely responsible for the reduction of $NaNO_2$-induced MHb observed. Frequently, the stress of saline or other vehicle injections are considered minor and inconsequential. However, in this study it generated significant effects, ones that would be thought relatively impervious to stress effects since they were a biochemical modification of the hemoglobin molecule. Furthermore, the effects of stress lasted 24 hours after treatment and may well have been detected at longer postinjection periods had tests been made at later times. Although the stress-induced mechanism responsible for the changes in MHb levels is unknown, it is natural to consider increased corticosterone levels as a likely candidate for the observed changes. Other researchers have reported an extended rise in corticosterone levels following a single PBS injection. A 300% increase above baseline was found 2 hr after injection (1). It has been discovered that the amount of corticosterone found in plasma was positively related to the apparent distress exhibited by animals at the time of injection (8). In addition, the simple handling of a rat reduced the behavioral effects of toxic levels of colchicine (18), possibly through a corticosterone-related mechanism. These results underscore the importance of including both vehicle control and "no treatment" control groups in experimental designs.

Pretreatment with either calcium antagonist 2 hr prior to $NaNO_2$ administration eliminated the PBS-induced reduction in MHb levels found on control animals after nitrite administration. In the groups receiving the calcium channel antagonists at this time (2 hr) before the nitrite, these drugs blocked this "saline effect." However, when the drugs were given 24 hr before the nitrite, the amount of MHb found in the blood was reduced relative to noninjected controls and to a degree nearly equivalent to that found in the saline-injected animals. Their MHb levels were significantly lower than those of animals that did not receive a prior injection. This suggests that the injection of the calcium channel antagonists did elicit a "stress" response but that the presence of the drug or a metabolite in the blood later blocked the expression of the effect on the MHb levels. Thus, the reduction of MHb was not found when the drugs were given 2 hr before the nitrite. When the drugs or metabolites had been eliminated, e.g., 24 hr later, the reduction of MHb presumably related to the stress of the injection procedure was observed.

The effects of the nimodipine or verapamil at 2 hr postinjection could be to reduce the intensity of the stress response or, possibly, to specific components of the stress reaction, e.g., the release of corticosterone, epinephrine, or other biological reactions to this form of stress. A related possibility is that the drug or one of its metabolites could induce the synthesis of MHb reductase, thus producing a more rapid reduction of the iron in the heme moiety to the ferrous state. MHb reductase synthesis could also be affected in a secondary fashion by the hormonal or other biological

sequelae of the stress response. In any of these cases, however, the reduction of the MHb levels produced by the saline pretreatment is likely to be calcium related since the two drugs used are quite different in their chemical structure and their side effects. Furthermore, it would appear that the actual presence of the drugs or their active metabolites is essential for the blocking of the saline-induced reduction of MHb, since this effect was not observed when the nitrite was given 24 hr after the administration of the calcium channel antagonists.

It should be noted that the effect of the calcium channel antagonists used in this study were found when using doses that have been commonly studied in the literature (5, 11–13). Overall, the amounts are on the low side relative to many experimental studies, although comparability is made difficult by the use of different vehicles and routes of administration.

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