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Dissociation of Dipsogenic and Depressor Responses Produced by Hypotensive Agents[1]

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FALK, J. L., S. FORMAN AND M. TANG. *Dissociation of dipsogenic and depressor responses produced by hypotensive agents.* PHARMAC. BIOCHEM. BEHAV. 1(6) 709–718, 1973. – Since the direct, vascular, smooth-muscle-relaxing agents isoproterenol and diazoxide produce substantial drinking responses in water-satiated rats, other direct-acting (sodium nitrite, sodium nitroprusside, hydralazine) and indirect (methyldopate) hypotensive drugs were administered to assess their dipsogenic efficacy. Although characteristic hypotensive dose-effect relations were observed for these drugs, no significant drinking occurred over the range of doses producing hypotension. Combinations of the three direct-acting agents with the alpha-adrenergic blocker tolazoline yielded moderate dipsogenic effects, although tolazoline alone was dipsogenically ineffective at these doses (1 and 10 mg/kg) as determined in a previous study. Thus, the ineffectiveness of the direct-acting agents as dipsogens was not due to inherent behavioral toxicity. Propranolol, which was shown previously to antagonize diazoxide-induced drinking did not antagonize the hypotensive effect of diazoxide. Since some of the drugs used increase plasma renin activity, but failed here to stimulate drinking, increased plasma renin activity may be an incomplete explanation of the dipsogenic activity of certain peripherally-acting agents.

Drinking Hypotensive agents Blood pressure Renin-angiotensin system Diazoxide
Sodium nitroprusside Sodium nitrite Methyldopa Hydralazine Tolazoline Propranolol

IN PREVIOUS studies it was shown that diazoxide, a hypotensive benzothiadiazine with antidiuretic actions, produced a severe, primary polydipsia in rats [6, 7, 8]. As part of a program aimed at delineating the dipsogenic action of diazoxide, several hypotensive agents were examined to determine if they too might induce water intake. Some drugs (sodium nitrite, sodium nitroprusside) were chosen because their hypotensive action is similar to that of diazoxide [4, 24, 28, 31, 34]. That is, they are direct, vascular, smooth-muscle-relaxing agents whose hypotensive action is not attributable to ganglionic blockade, catecholamine depletion, diuretic or central nervous actions. Other drugs were selected primarily on the basis of whether they stimulated (hydralazine and also sodium nitroprusside) or inhibited (methyldopate) renin release, as well as possessing either direct (hydralazine) or indirect (methyldopate) hypotensive action [2, 13, 14, 21, 22, 26, 27, 33].

Recent experiments have demonstrated that the dipsogenic action of diazoxide is antagonized specifically by propranolol, a beta-adrenergic blocking agent [8]. In order to determine whether this antagonism was dependent upon some reversal of diazoxide-induced hypotension, this drug combination was repeated and blood pressure was monitored.

If a particular drug fails to produce a dipsogenic response this could occur either because the agent is simply not a dipsogen or because it might produce behaviorally toxic side effects which prevent the occurrence of drinking (e.g., malaise). For this reason, if we suspected that drug-induced malaise might interfere with drinking, the particular agent also was given in combination with the weak dipsogen tolazoline (an alpha-adrenergic blocker) to determine whether the animals would indeed respond dipsogenically while stimulated by the agent in question.

EXPERIMENT 1: HYPOTENSIVE AGENTS AND DIPSOGENIC RESPONSE

Method

Animals. Twenty-four male, albino rats (Holtzman strain) with a mean body weight of 323 g (range: 227–435 g) were used. They were housed individually in a temperature-controlled room with a 12-hr light–dark cycle.

Intake schedule. All animals were adapted to the following feeding-drinking schedule for 3–6 days. Water was always available from a calibrated Richter tube and food was removed only for a 3-hr period during the day. Normally, little food or water is consumed during this part of the light cycle. This water-intake period with a low mean and variance allows even small drug-induced increases to be detected. During the 3-hr period, hourly water intake was measured. Then food (Purina Laboratory chow, pelleted)

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was replaced. At the beginning of the 3-hr period, animals were weighed and any drug or control injection administered. Thus, the effect of various drug doses on 3-hr water intake in the absence of concomitant food intake was evaluated.

Drugs. All drugs were dissolved in isotonic saline and administered subcutaneously (SC) in volumes less than 0.8 ml. The following drugs were used: Sodium nitroprusside (Sigma Chemical Co.), sodium nitrite (Fisher Sci. Co.), hydralazine HCl (Sigma Chem. Co.), tolazoline HCl (Priscoline, generouly supplied by Dr. A. J. Plummer, Ciba Phaarmaceutical Co., Summit, N. J.), and methyldopate HCl (a gift from Merck, Sharp and Dohme Research Lab, Rahway, N. Y.). All doses are specified as the weight of the salt.

Procedure. The 24 animals were divided into four groups of six corresponding to four drug dose-effect studies. Within each group, animals were given one of three drug dose levels or a saline control injection on each treatment day in random order. The four drugs used were sodium nitrite (20, 40, and 60 mg/kg), sodium nitroprusside (1, 2, and 4 mg/kg), hydralazine (10, 20, and 30 mg/kg), and methyldopate (25, 50, and 100 mg/kg). On non-injection days animals remained on the intake schedule and 2 6 days elapsed between injections.

Upon completion of the above phase, the median dose of three of the drugs was combined with either a 1 or 10 mg/kg tolazoline injection (SC). Tolazoline was given 15 min before the other drug. In each group, 3 animals received the 10 mg/kg tolazoline dose plus the other drug as the first treatment and then received the 1 mg/kg tolazoline dose plus the drug 3–4 days later. The other 3 animals were treated in the reverse order.

Results

Figure 1 shows the mean 3-hr water intakes following injections of three of the drugs. For all three drugs the magnitude of the drinking was small. Analyses of variance (one-way, repeated measures design, [35], p. 105) performed on the sodium nitrite and sodium nitroprusside groups yielded insignificant F-values. The overall F for the hydralazine group was significant ($F = 4.62$, $df = 3, 15$, $p<0.05$). However, since the animals were depressed at the largest dosage (30 mg/kg), an analysis of variance was performed excluding this dosage point. The resulting F-value of 2.25, $df = 2, 10$, was not significant ($p>0.1$). Therefore, the significant overall F-value for hydralazine would seem to be largely due to the depressive effect of the largest dose, rather than any dipsogenic effect of the lower doses. Hence, none of the three hypotensive agents evoked a dipsogenic response.

Figure 2 shows that when the median dose of the three hypotensive agents was combined with 1 or 10 mg/kg tolazoline moderate drinking resulted. The 3-hr water intake for each hypotensive agent alone was tested for statistical significance against the intake induced by its combination with the two dose levels of tolazoline. Sodium nitrite in combination with 10 mg/kg tolazoline produced greater water intake than sodium nitrite alone ($p<0.025$). Sodium nitroprusside was synergized by both 1 mg/kg ($p<0.01$) and 10 mg/kg ($p<0.005$) tolazoline. When hydralazine was combined with 10 mg/kg tolazoline as the first drug combination treatment all three animals died. Therefore, the drug-combination data shown for hydralazine are for the remaining animals which received the tolazoline in the 1 10 mg/kg order. While Fig. 2 shows a sizable increase over the 20 mg/kg hydralazine drinking level when 1 mg/kg tolazoline was given in combination with the hydralazine, the small number of animals involved does not permit a valid significance test.

Methyldopate did not produce water intake in excess of that seen under saline control conditions at any of the dose levels given.

EXPERIMENT 2: HYPOTENSIVE AGENTS: TIME COURSE OF EFFECTS

While the drugs used in Experiment 1 are all known hypotensive agents, it is possible that the general lack of dipsogenic efficacy could be attributable to inappropriate dosage or temporal onset with respect to the hypotensive response. In order to assess these drugs and compare them with the known hypotensive, dipsogenic effect of diazoxide, the present experiment was undertaken.

Method

Animals. Twelve male, albino rats (Holtzman strain) with a mean body weight of 326 g (range: 274 378 g) were used. They were housed as in Experiment 1.

Blood pressure determination. Indirect blood pressure was measured with a tail-cuff method. The complete system was composed of components manufactured by Narco Bio-Systems, Inc., Houston, Texas. Rats were adapted to light restraint in a plastic cage which had a temperature-controlled base for gentle heating to induce vasodilation. A pressure cuff was placed at the base of the tail and was inflated up to 200 mm Hg and deflated at a rate of 10 mm Hg/sec by a programmable electro-sphygmomanometer. A pulse transducer fastened distal to the cuff recorded pulses and the point of pulse reappearance during cuff deflation was taken as the systolic pressure. The pulse and pressure levels were recorded simultaneously on a Physiograph. Several determinations per day were taken, and with gentle handling reliable day-to-day blood pressures were recorded.

Drugs. Sodium nitroprusside and sodium nitrite were used as per Experiment 1. Diazoxide (3-methyl-7-chloro-1, 2, 4-benzothiadiazine-1, 1-dioxide), generously supplied by Schering Corp., Bloomfield, N. J. (Hyperstat), was dissolved in 1 N NaOH and isotonic saline. The least amount of NaOH solution was used for the particular dose level which would allow the drug to be dissoved. The ratio of 1 N NaOH to isotonic saline for the 3 dose levels of diazoxide was: 1:16 for 20 mg/kg, 1:7 for 40 mg/kg, and 1:3 for 80 mg/kg diazoxide. Diazoxide solutions were prepared immediately prior to injection. Propranolol hydrochloride was dissolved in isotonic saline. All drugs were administered SC immediately prior to placing the animal into the blood pressure recording situation, except propranolol which was administered 15 min prior to diazoxide.

Experimental design. Three animals were assigned to each group which consisted of a particular drug or drug combination. The animals were adapted for 4 weeks to the blood pressure determination situation in which their pressures were recorded 3 times per week. Dose-effect relations were determined for certain drugs, with at least 7 days elapsing between doses. Two of the drugs and their dose levels used in Experiment 1 were administered in this experiment (sodium nitroprusside, sodium nitrite), as well

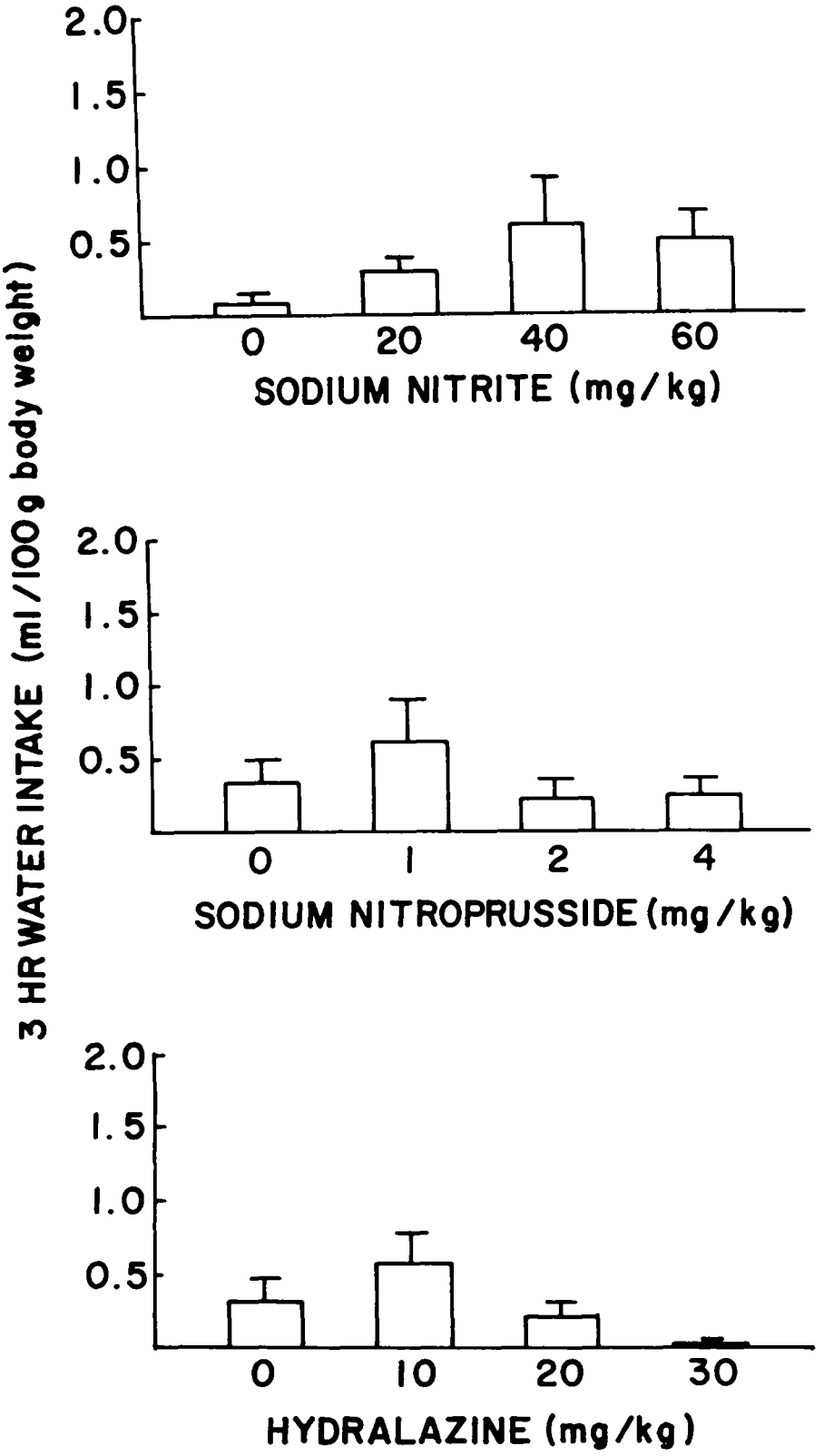

FIG. 1. Mean (+ S.E.) 3-hr water intake of satiated rats following saline control or drug injection (SC).

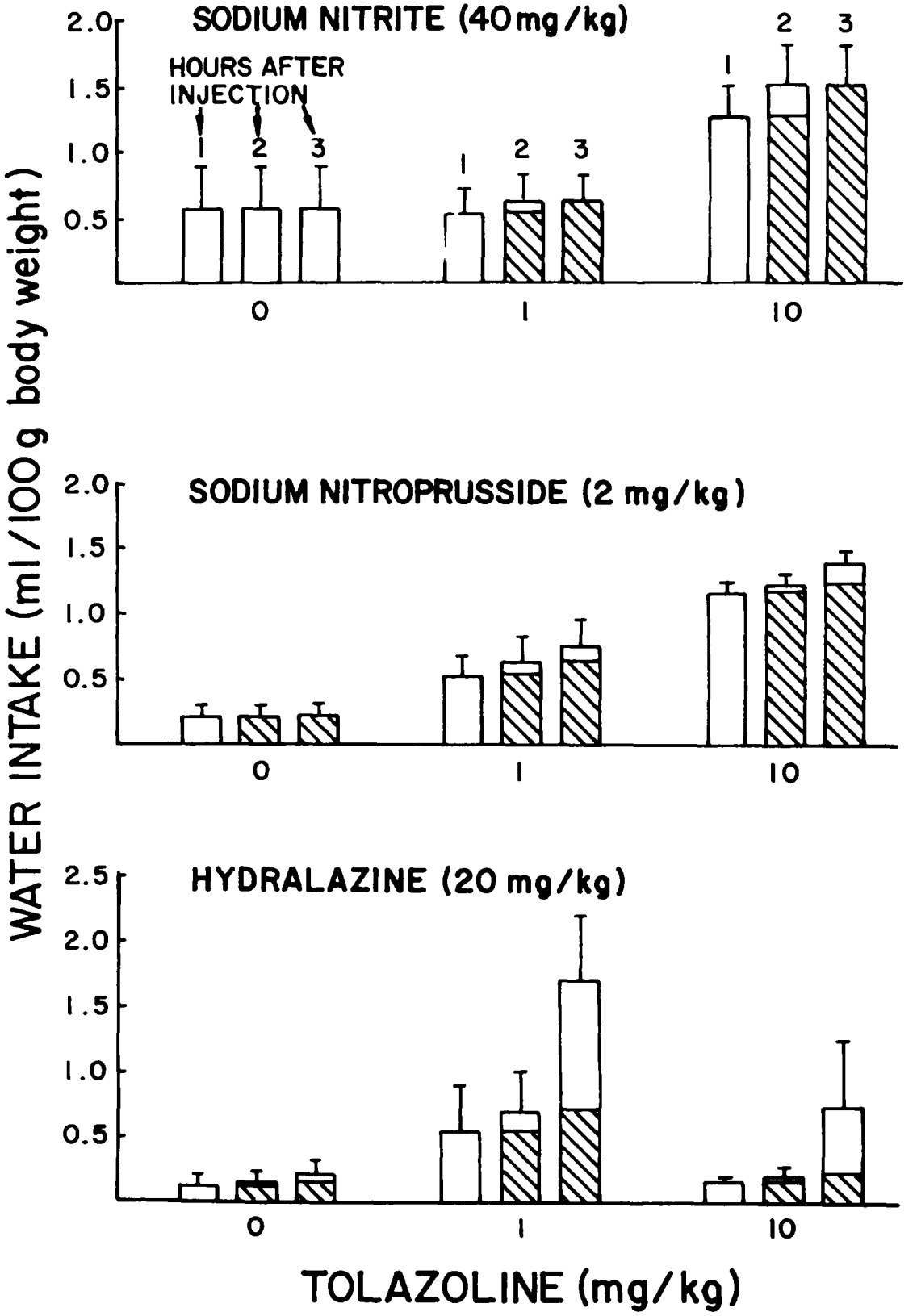

FIG. 2. Mean (+ S.E.) cumulative 3-hr water intake of satiated rats given one of 3 hypotensive drug injections (SC) in combination with saline control, 1 or 10 mg/kg tolazoline injected 15 min previously.

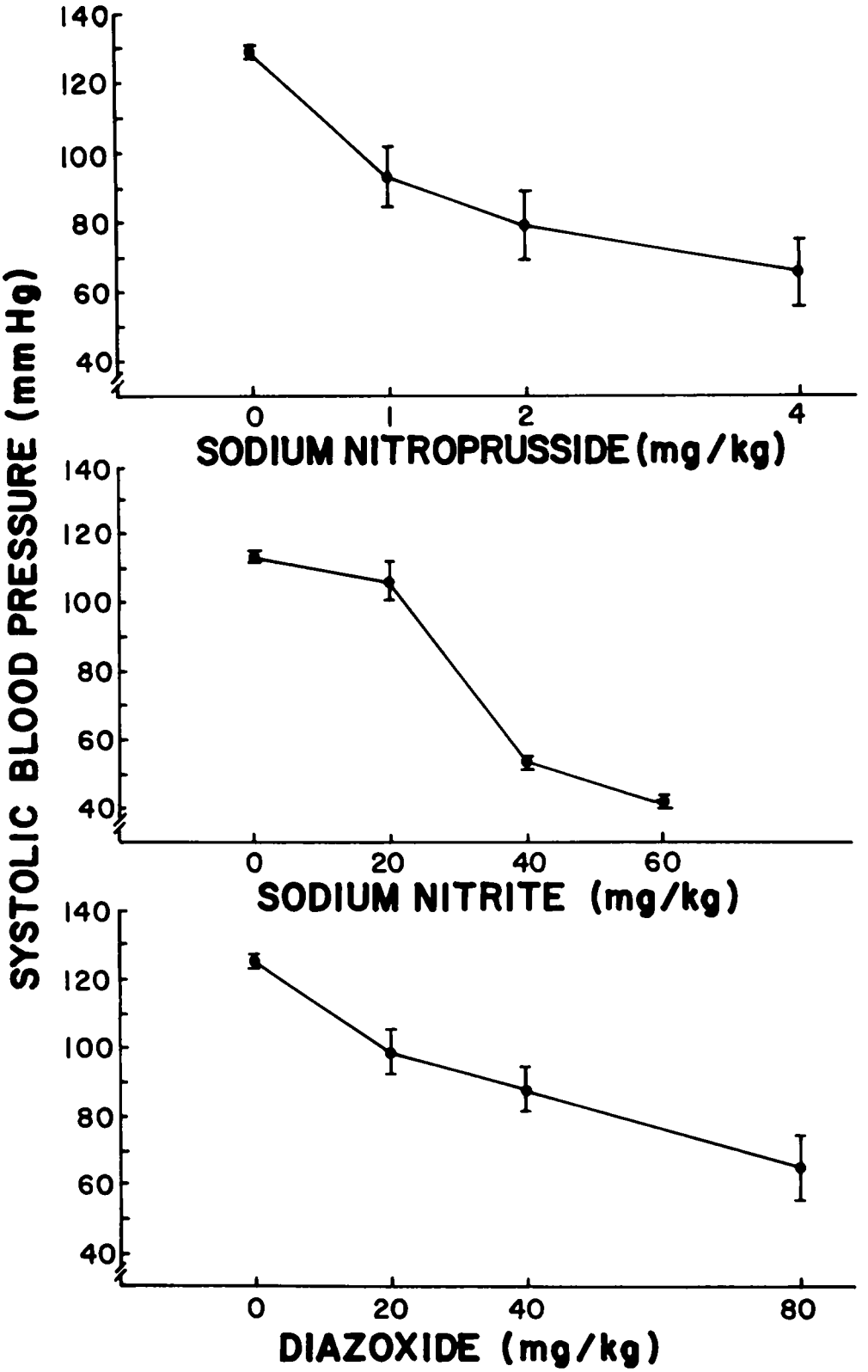

FIG. 3. Mean blood pressure (±S.E.) of rats given 3 dose levels of 3 hypotensive agents. Plotted points are the means of the minimum pressures recorded within 1 hr post-injection (SC). N = 3 rats for each drug.

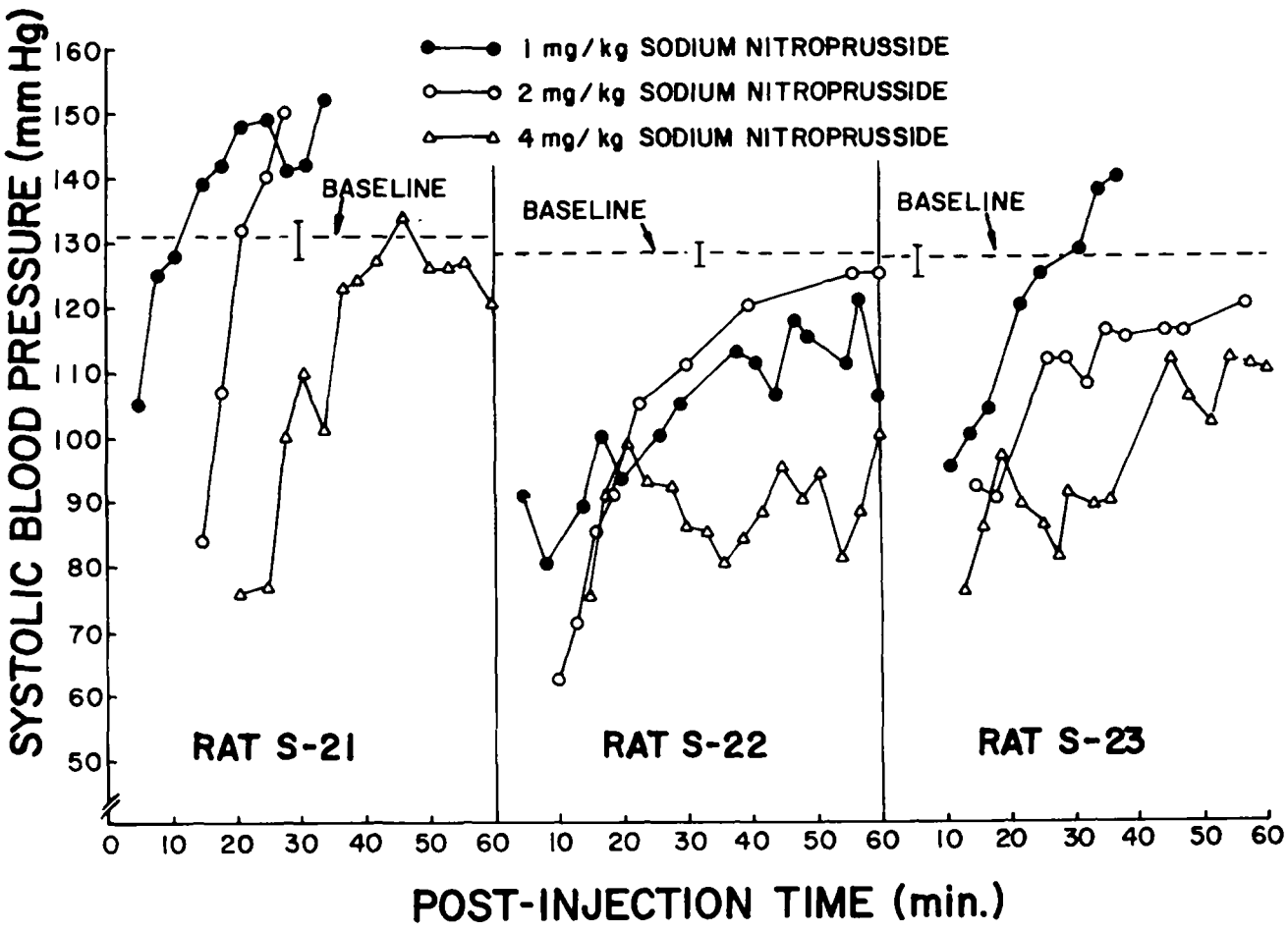

FIG. 4. Blood pressure changes for 3 rats following SC injection of sodium nitroprusside at 0 min.

as diazoxide and diazoxide-propranolol combinations. All dose levels were administered to each animal in a nonsystematic order within each group.

Results

Figure 3 shows that all three drugs yielded hypotensive effects and that the range of hypotension was roughly similar. Figures 4, 5, and 6 delineate the time course of the hypotensive effects over a one-hr period. The hypotensive effect of sodium nitroprusside occurred with short latency but was also of short duration. Figure 4 shows that even at the largest dose, the blood pressure had recovered or was on the way to recovery after one hour. However, for sodium nitrite and diazoxide, doses which were hypotensive remained so during the entire one-hour blood pressure monitoring period (Figs. 5 and 6). As shown by Fig. 7, propranolol alone had no hypotensive effect, nor did it antagonize the hypotensive effect of diazoxide. In fact at 10 mg/kg, propranolol appears to produce a modest hypertensive effect.

DISCUSSION

Isoproterenol [16] and diazoxide [6, 7, 8] are both agents with marked efficacy for producing water intake when injected peripherally, even though they are both antidiuretics. Since both agents derive their hypotensive action from direct relaxation of vascular smooth muscle, additional drugs having this direct vasodilatory effect were tested for possible dipsogenic efficacy. Sodium nitrite, sodium nitroprusside and hydralazine, although direct vasodilators [4, 24, 28, 29, 30, 34], failed to yield significant drinking in the present study. However, the combination of each of the drugs with tolazoline produced moderate drinking responses, demonstrating that the three direct vasodilators were not simply incapacitating the animals with respect to weak dipsogenic effects. Tolazoline alone did not evoke a drinking response [8] at the doses used in the present experiment (1 and 10 mg/kg), but some drinking was noted when a large dose of 17 mg/kg was given [16]. It is of interest that tolazoline in the present dose range acted synergistically with all three of the direct vasodilators to evoke some drinking. It also acted synergistically to increase diazoxide-induced drinking [8].

It has been proposed that the mechanism by which beta-adrenergic and certain other agents evoke drinking is by the release of renin [11, 12, 19, 20]. Presumably, the released renin acts upon plasma renin substrate to increase the circulating level of angiotensin, a proven dipsogenic agent [9]. Renin release can occur by direct, beta-adrenergic stimula-

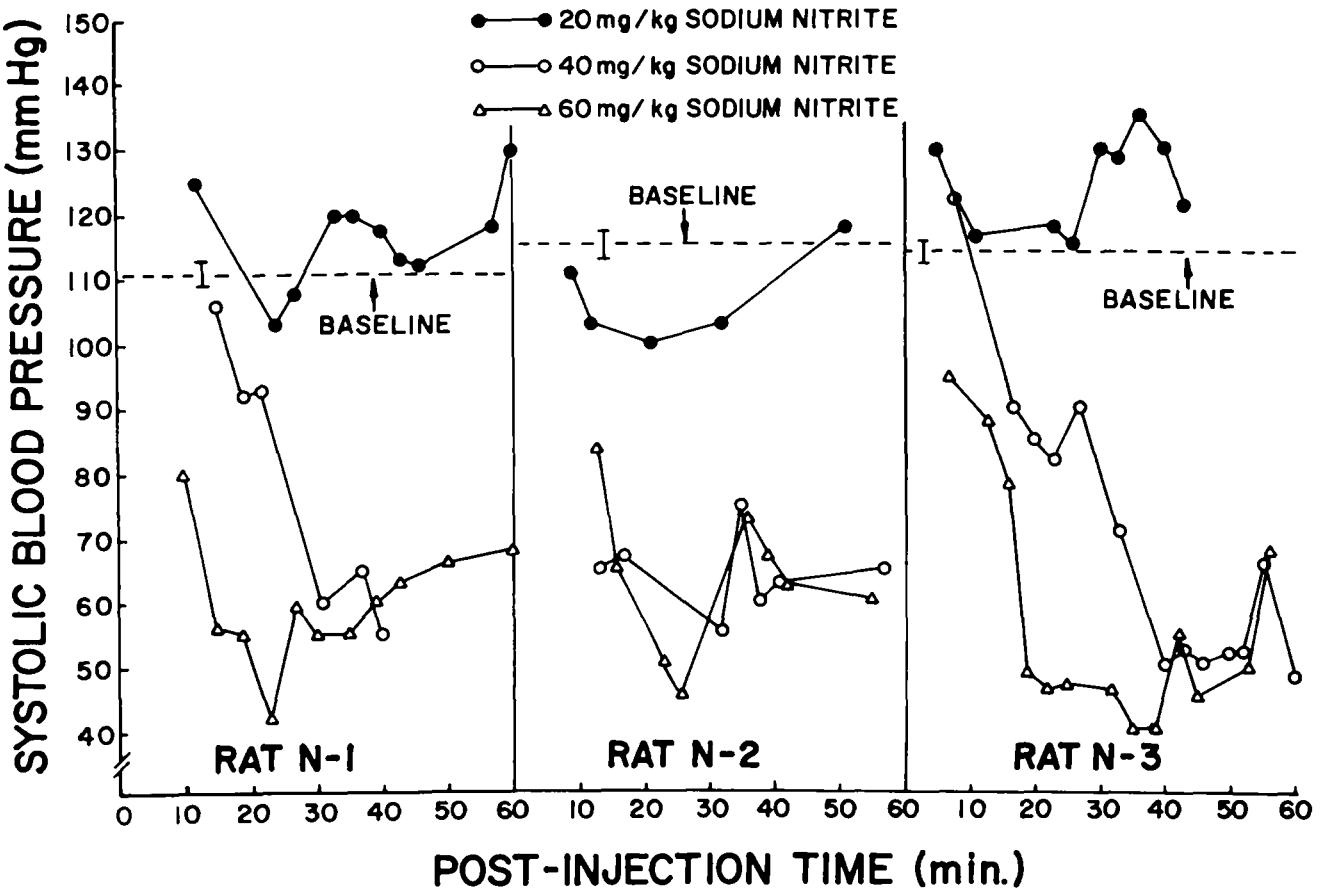

FIG. 5. Blood pressure changes for 3 rats following SC injection of sodium nitrite at 0 min.

tion of renal receptors, or indirectly as a sympathetic response to various cardiovascular and pharmacological stimuli [1,10]. While there is evidence correlating these two modes of renin release with the evocation of drinking [20], and studies demonstrating that nephrectomy abolished the drinking induced by isoproterenol [11, 12, 19], phentolamine [19], hydralazine plus bretylium [19], lithium [11] and diazoxide [8], the present experimental results cannot offer unequivocal support to an explanation completely dependent upon the renin-angiotensin system. There was a failure to find any dipsogenic response to hydralazine alone, and the response which has been reported [25] was of small magnitude (2 -4 ml). However, hydralazine stimulated renin release in the rat as efficaciously as isoproterenol [26] and the present experiment covered the appropriate dose range. It also increased plasma renin activity in normotensive and hypertensive humans [33] and to a moderate extent in dogs [2]. Likewise, sodium nitroprusside alone failed to evoke drinking, but has been shown to release renin in the dog [2, 5, 32] and in man [13,14].

Neither the direct hypotensive nor the renin-releasing actions of the various drugs, then, seem able to predict the presence or degree of the dipsogenic response. Thus, although diazoxide and isoproterenol are both dipsogens [7,16] and depressor, renin-releasing agents [1, 3, 15, 18, 26, 36], this correlation may not reflect the mechanism of action of their dipsogenic property.

Both hydralazine and methyldopa produce increases in renal blood flow [17, 23, 30] even though they are hypotensive agents. But while hydralazine increases plasma renin activity [2, 26, 33], methyldopa decreases it [21, 22, 27]. Nevertheless, neither agent alone is dipsogenic. Perhaps some endocrine concomitant of a reduced renal blood flow is more important in producing a dipsogenic response than the accompanying plasma renin activity changes.

It is clear that the dipsogenic property of diazoxide is unrelated to the hypotensive effect per se. Not only do several other hypotensive agents fail to evoke drinking, but the antagonism of diazoxide-induced drinking by propranolol [8] cannot be explained by any accompanying antagonism of the hypotension. Propranolol did not alter the course of diazoxide-induced hypotension in the present study, even though it has some pressor action in the rat [37,38]. This pressor action is evident at 10 mg/kg (cf. Fig. 7).

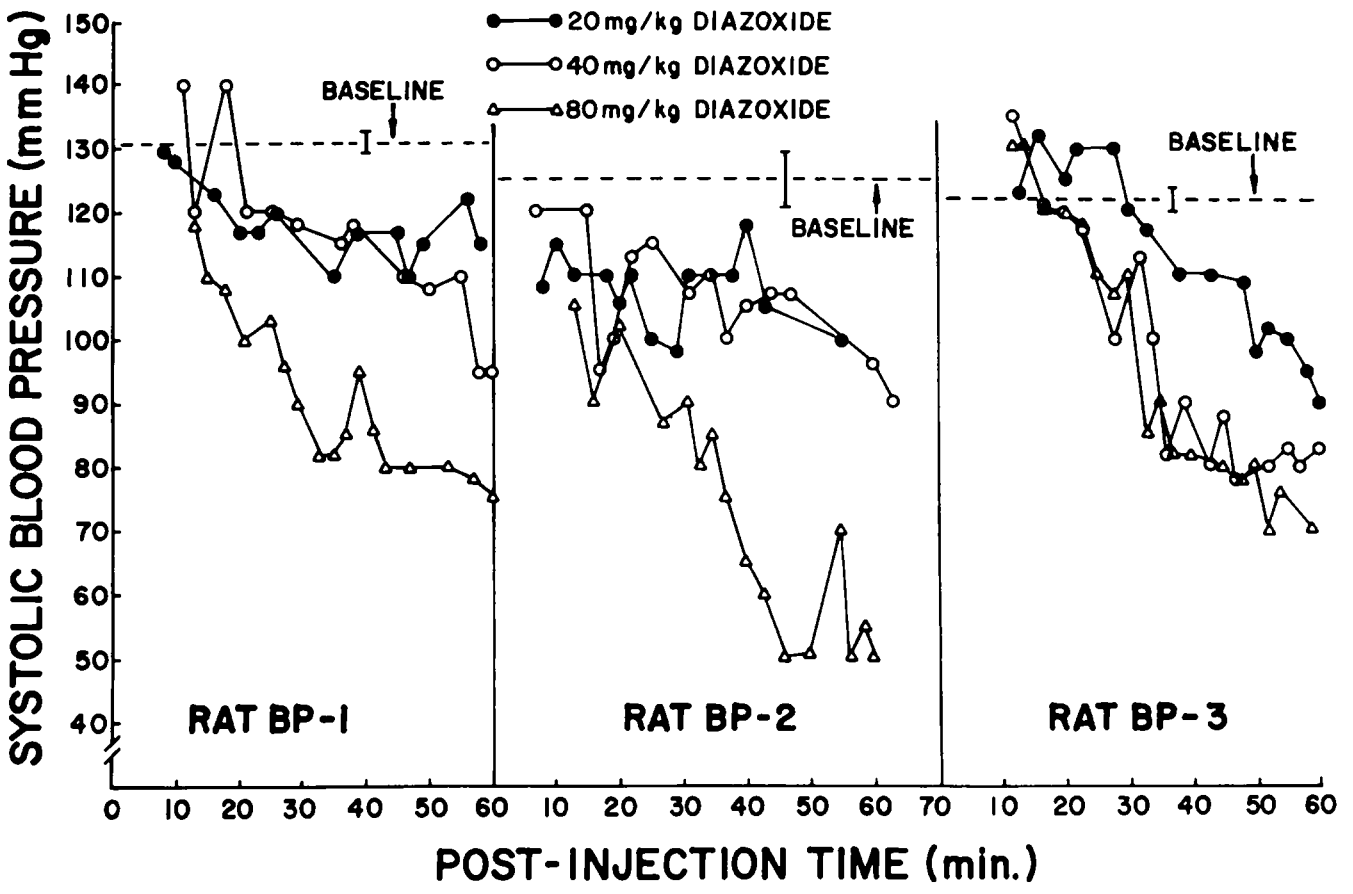

FIG. 6. Blood pressure changes for 3 rats following SC injection of diazoxide at 0 min.

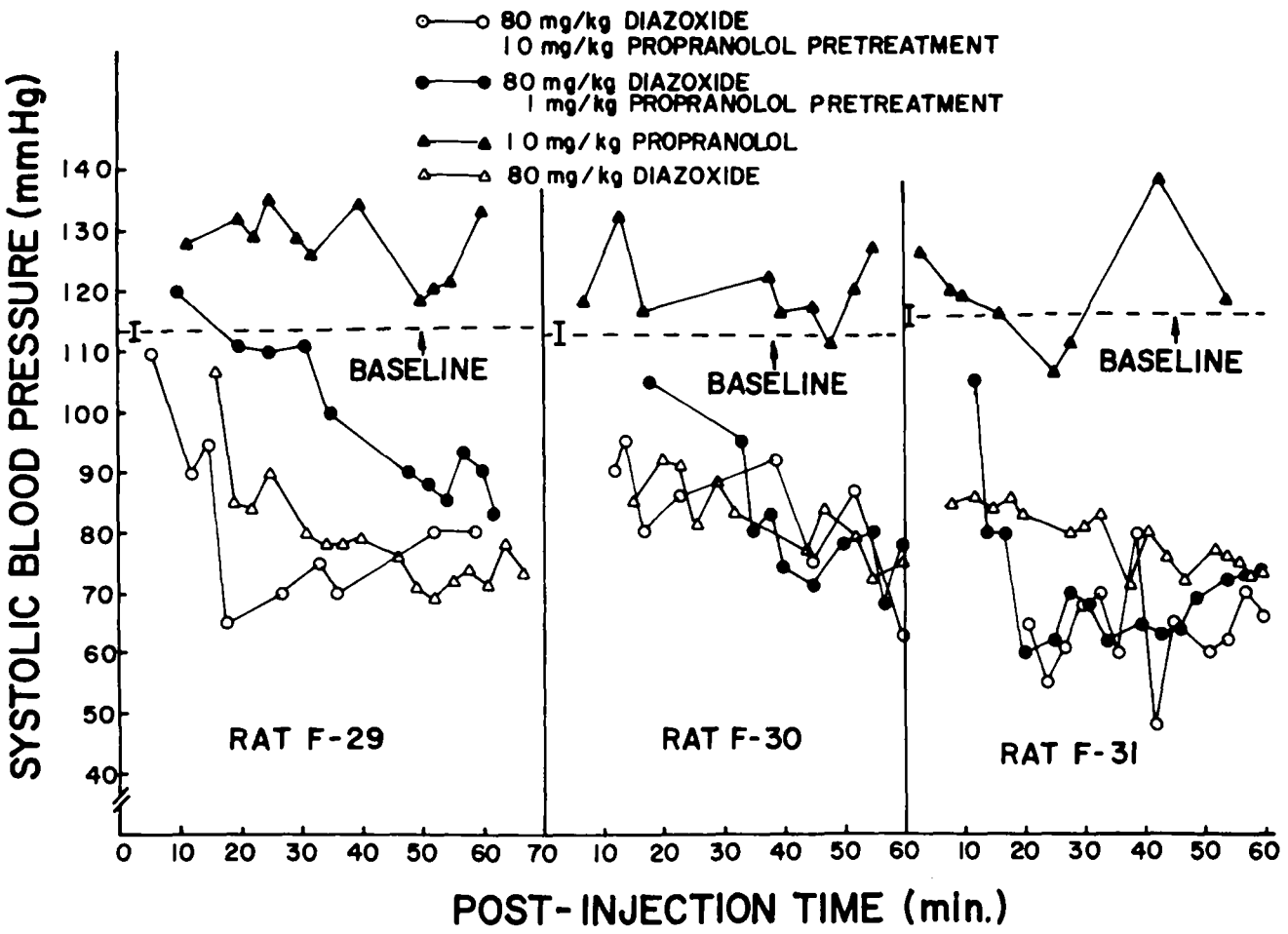

FIG. 7. Blood pressure changes for 3 rats following SC injection of diazoxide, propranolol, or combinations of propranolol and diazoxide.

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