Cardiovascular Effects of Isoxsuprine and Sotalol

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Abstract □ Isoxsuprine, a vasodilating agent, and sotalol, a β-adrenergic receptor blocking agent, were administered separately and in combination to normotensive rats and hypertensive dogs. In rats, sotalol produced a hypotensive response only in low doses whereas isoxsuprine produced a significant hypotension at all doses used. Administration of either agent was followed by a significant decrease in heart rate. The combination of isoxsuprine with sotalol decreased the heart rate more than either drug alone, but the hypotensive response did not follow this pattern. In hypertensive dogs, isoxsuprine and sotalol significantly decreased blood pressure. In contrast to the rat, the hypotensive response to isoxsuprine in the dog was accompanied by an increase in heart rate. The combination of isoxsuprine with sotalol produced a greater hypotensive response than either drug alone in medium and high doses but not in low doses. Sotalol prevented the increase in heart rate that accompanied the hypotensive response to isoxsuprine. The data suggest that the combination of isoxsuprine and sotalol may be useful in the treatment of hypertension.

Keyphrases □ Isoxsuprine and sotalol—cardiovascular effects after separate and concurrent administration in rats and dogs, potential antihypertensive therapy □ Sotalol and isoxsuprine—cardiovascular effects after separate and concurrent administration in rats and dogs, potential antihypertensive therapy □ Antihypertensive activity—concurrent administration of isoxsuprine and sotalol evaluated, rats, dogs □ Cardiovascular effects—isoxsuprine and sotalol, separate and concurrent administration, rats, dogs

The treatment of essential hypertension has centered in recent years on the use of various agents that interfere with adrenergic neuronal transmission. Although this approach has been generally successful in reducing blood pressure, it is not without problems. Hypotensive agents that interfere with sympathetic nervous function commonly cause postural hypotension, weakness, lethargy, and sexual dysfunction (1–6).

Since an elevation of peripheral vascular resistance appears to be the major hemodynamic abnormality present in essential hypertension (7), a new treatment based on the concurrent administration of vasodilating and β-receptor blocking drugs was recently introduced (8, 9). In previous investigations, drugs such as hydralazine were used to produce vasodilation (9). Hydralazine was reported to decrease peripheral resistance by a direct relaxing action on arteriolar smooth muscle (10–12). Its antihypertensive effectiveness is limited, however, by a reflex action that increases heart rate and cardiac output (12, 13). Even though the reflex cardiac augmentation produced by hydralazine can be prevented by propranolol (9), other side effects, such as immunologic disturbances occurring during chronic therapy (14), make the use of different vasodilating drugs more desirable.

Isoxsuprine[1], an agent that produces vasodilation through a direct relaxing action on vascular smooth muscle (15–17) as well as by activation of β-adrenergic receptors (18, 19), appears to be more suitable for this purpose since it has been reported to be free of undesired effects (20). The hypotensive effect produced by isoxsuprine may be useful in the treatment of hypertension if the positive chronotropic and inotropic effects accompanying the hypotensive response can be prevented. In the present investigation, sotalol[2], a β-receptor blocking agent, was utilized in an attempt to block selectively the cardiac augmentation produced by isoxsuprine while retaining the peripheral vasodilating and hypotensive effects.

[1] Vasodilan, Mead Johnson.

EXPERIMENTAL

Measurement of Blood Pressure and Heart Rate in Anesthetized Rats—Wistar albino rats of both sexes, weighing approximately 200–250 g., were anesthetized with 1.25 g./kg. of urethan administered intraperitoneally. The animals were secured ventral side up on an animal board. A midline incision was made over the tracheal region; the trachea was exposed and cannulated with polyethylene tubing[3] to ensure freedom of respiration. A common carotid artery was isolated and cannulated with polyethylene tubing[4], which was connected to a pressure transducer[5]; all recordings were made on a polygraph[6]. One femoral vein was isolated for injection purposes, and the blood pressure was allowed to stabilize before administration of any drug. In each case the experimental compounds, dissolved in saline, were administered in volumes of 0.2 ml. or less. Groups of three male and three female rats were administered intravenously either 2, 3, or 4 mg./kg. of isoxsuprine, sotalol, or equal combinations of both. The heart rate and systolic blood pressure were monitored for 90 min. following drug administration. The changes obtained after drug administration were compared to the control values obtained before drug administration and analyzed by the Student t test. Values of $p \leq 0.05$ were considered significant.

Measurement of Blood Pressure and Heart Rate in Unanesthetized Hypertensive Dogs—Two male and two female mongrel dogs were made hypertensive by the method of Grollman (21). In this study the method was modified in that both kidneys were ligated, thus increasing the amount of residual, normally functioning renal tissue and minimizing the possibility of uremia developing. Two mongrel dogs who were received from the supplier and found to be hypertensive were used as well. Systolic blood pressure was determined before surgery and at weekly intervals thereafter until a stable pressure of 180 mm. Hg or higher was attained. All dogs were given normal saline for drinking water *ad libitum*. The dogs were trained to lie quietly in a hammock, which allowed all four limbs to be freely suspended. The blood pressure and heart rate were recorded from the brachial artery by means of an infant sphygmomanometer cuff which contained a sensitive microphone[7]. The cuff was connected to an electrosphygmomanometer, and recordings were made on a physiograph[8]. Ten clear readings of the systolic blood pressure were recorded at each time interval, and an average value was obtained. Heart rate was determined from the pulse wave by partially occluding the artery for 15 sec.

Systolic blood pressure and heart rate were determined 5–10 min. before administration and at 30, 90, 180, and 360 min. after administration. Each dog served as its own control. All drugs were administrated orally as a powder in gelatin capsules. Each dog received nine drug dosages, and the schedule of administration and

[2] 4′-[1-Hydroxy-2-(isopropylamino)ethyl]methanesulfonanilide monohydrochloride, MJ-1999, Mead Johnson.
[3] PE 240.
[4] PE 90.
[5] Statham.
[6] Grass 5D.
[7] Korotkoff
[8] E&M Mark IV console.

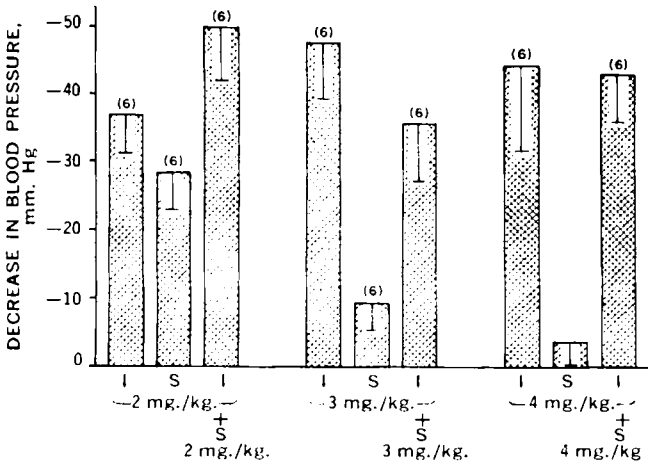

Figure 1—*Effect of isoxsuprine (I) and sotalol (S) on blood pressure in rats. Isoxsuprine and sotalol were administered intravenously alone and in combination (I + S). The number of observations is indicated in parentheses. The hypotensive response produced by I is not significantly different from I + S at any dose. The response to I + S is significantly greater than that to S at the 3- and 4-mg./kg. doses* ($p < 0.05$).

dosage was as follows: isoxsuprine at 6, 12, and 18 mg./kg.; sotalol at 10, 20, and 30 mg./kg.; and a simultaneously administered combination of 6, 12, and 18 mg./kg. of isoxsuprine with 10, 20, and 30 mg./kg. of sotalol, respectively. These doses of sotalol were reported by Lish *et al.* (22) to produce β-receptor blockade in dogs as measured by blockade of isoproterenol-induced tachycardia. A control series was performed in which lactose was administered in place of the active drugs, and the heart rate and blood pressure were followed over a time course identical to that of the drug studies. Each dog was given at least a 3-day rest between drug administrations. The effects of the drugs administered separately and in combination were analyzed by the Student *t* test. Values of $p \leq 0.05$ are considered significant.

RESULTS

Effect of Isoxsuprine and Sotalol on Blood Pressure and Heart Rate in Anesthetized Rats—The intravenous administration of isoxsuprine in rats produced a significant decrease in blood pressure ($p < 0.001$; Fig. 1). There was no significant difference in the magnitude of the hypotensive response between the three doses of isoxsuprine ($p > 0.05$). The decrease in blood pressure produced by isoxsuprine was not accompanied by a reflex increase in heart rate (Fig. 2). In fact, in all but two instances (2/18), the rats responded with a bradycardia, the magnitude of which was not significantly different between the three doses of isoxsuprine ($p > 0.05$).

The administration of the low dose (2 mg./kg.) of sotalol resulted in a significant decrease in blood pressure ($p < 0.05$), while the medium (3 mg./kg.) and high (4 mg./kg.) doses did not produce a significant depressor effect ($p > 0.05$; Fig. 1). The administration of sotalol produced a significant decrease in heart rate at all three doses employed ($p < 0.05$; Fig. 2). There was no significant difference in the magnitude of the effect at the different doses.

The combination of isoxsuprine and sotalol produced a significant hypotensive effect at all three dose levels ($p < 0.05$; Fig. 1). Although the hypotensive response produced by the combination was significantly greater than that produced by sotalol alone at the same dose ($p < 0.05$), it did not differ from that produced by isoxsuprine alone at the same dose ($p > 0.05$). Thus, there was no evidence to suggest that sotalol potentiated the hypotensive response produced by isoxsuprine. The combination of isoxsuprine with sotalol produced a greater negative chronotropic effect than the administration of either compound alone in the low and medium doses ($p < 0.05$) but not at the high dose ($p > 0.05$).

Effect of Isoxsuprine and Sotalol on Blood Pressure and Heart Rate in Unanesthetized Hypertensive Dogs—The oral administration of lactose to six unanesthetized hypertensive dogs did not produce any significant change in blood pressure or heart rate. The oral administration of isoxsuprine alone at low (6 mg./kg.), medium (12 mg./kg.), and high (18 mg./kg.) doses to hypertensive dogs produced a significant reduction in blood pressure of approximately 18–23 mm. Hg ($p < 0.05$; Fig. 3). There was no significant difference in the magnitude of the hypotensive effect between the three doses of isoxsuprine ($p > 0.05$). At each dose, a significant increase in heart rate accompanied the hypotensive response, the pattern being such that the high dose of isoxsuprine produced the greatest increase in heart rate (Fig. 4). The progressive elevation in heart rate produced by increasing the dose of isoxsuprine may explain the lack of a greater hypotensive response when the higher doses of isoxsuprine were administered.

The administration of sotalol produced a significant reduction in blood pressure, the magnitude of which was not different between the doses or from that produced by isoxsuprine at any dose (Fig. 3). However, the hypotensive response to sotalol, in contrast to isoxsuprine, was not accompanied by an increased heart rate but by a significant reduction in rate at each dose (Fig. 4). The magnitude of the effect was not different between doses of sotalol ($p > 0.05$). Diminution of sympathetic influences to the heart probably explains the hypotensive response to sotalol and the lack of any reflex adjustments.

The simultaneous adminstration of isoxsuprine and sotalol produced a consistent drop in blood pressure (Fig. 3). The maximum hypotensive response was achieved with the combination of 12 mg./kg. isoxsuprine and 20 mg./kg. sotalol (medium dose); increasing the dose of either drug did not result in a greater hypotensive response. Since the medium and high doses of the combination produced a decrease in blood pressure that was the same as the total of both drugs alone, the hypotensive effects of isoxsuprine and sotalol seem to be additive. It is possible that isoxsuprine in low doses (6 mg./kg.) produces vasodilation primarily through stimulation of β-receptors whereas higher doses produce a direct vasodilation which is independent of β-receptor activation. The β-receptor-mediated hypotensive response produced by low doses can be prevented by sotalol, but the direct vasodilation produced by high doses is not affected by sotalol.

The simultaneous administration of sotalol with isoxsuprine prevented the increase in heart rate produced when isoxsuprine was administered alone (Fig. 4). In the low and medium doses of the combination, sotalol converted the increase in heart rate to a decrease of approximately 16 beats/min. With the high dose of the combination, however, although there was an overall decrease in heart rate for the group, three of the six animals responded to the combination with an increase in heart rate which was, on the average, approximately 12 beats/min. It is possible, therefore, that the increase in heart rate in these animals may explain the lack of an increased hypotensive response with the high dose of the combination compared to the medium dose in which all dogs responded with a decrease in heart rate.

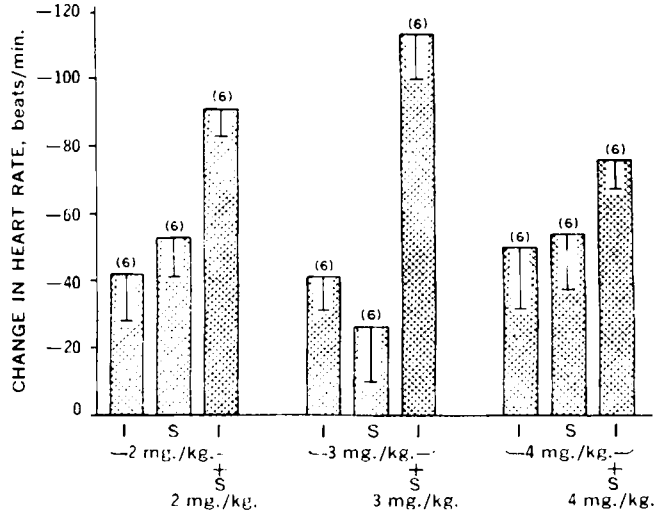

Figure 2—*Effect of isoxsuprine (I) and sotalol (S) on heart rate in rats. The response to I + S is significantly greater than the response to I or S alone at the 2- and 3-mg./kg. doses* ($p < 0.05$) *but not at 4 mg./kg. See Fig. 1 for details to legend.*

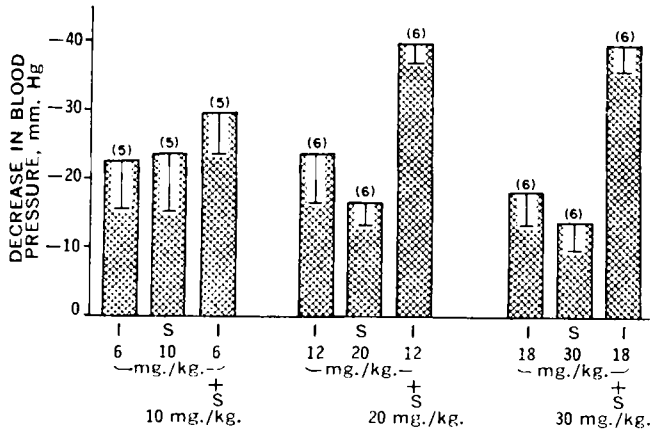

Figure 3—*Effect of isoxsuprine (I) and sotalol (S) on blood pressure in hypertensive dogs. Isoxsuprine and sotalol were administered orally alone and in combination (I + S). The number of observations is indicated in parentheses. The response to I + S is significantly greater than the response to I or S alone at the medium and high doses ($p < 0.01$). The response to I + S is the same as that to I or S at the low dose.*

DISCUSSION

The results of this study demonstrated that isoxsuprine is an effective hypotensive agent. Furthermore, the increase in heart rate produced by either a direct or reflex action of isoxsuprine could be prevented by the concurrent administration of sotalol whereas the hypotensive response was unaffected.

Marked differences were found in the heart rate effects of isoxsuprine in the conscious dogs and anesthetized rats. In the conscious dogs, the fall in arterial blood pressure produced by isoxsuprine was accompanied by an increase in heart rate, whereas in the rats the fall in blood pressure was always associated with bradycardia. Similar results were reported by Brunner *et al.* (23) using hydralazine as the vasodilating agent. These authors reported that the depressor response to hydralazine was accompanied by an increase in heart rate in conscious rats but a decrease in heart rate in anesthetized rats. It is concluded from the results of the present study that anesthetized rats must be used with caution in the evaluation of antihypertensive compounds where reflex cardiovascular changes may result since the anesthetized rat may obscure these changes. The present results in the unanesthetized dogs are not in agreement with Brunner *et al.* (23, 24), who reported that the administration of pronethalol prevented the direct vasodilating response to hydralazine in unanesthetized dogs. However, these results are in agreement with the findings of Gilmore *et al.* (25), Sonnerstedt *et al.* (26), Gottlieb *et al.* (8), and Zacest *et al.* (9), who found that the administration of β-blocking agents along with direct acting peripheral vasodilating agents prevents the reflex cardiac effects in response to a decrease in blood pressure but maintains or potentiates the hypotensive response. This study represents the first report of the use of isoxsuprine and sotalol for this purpose.

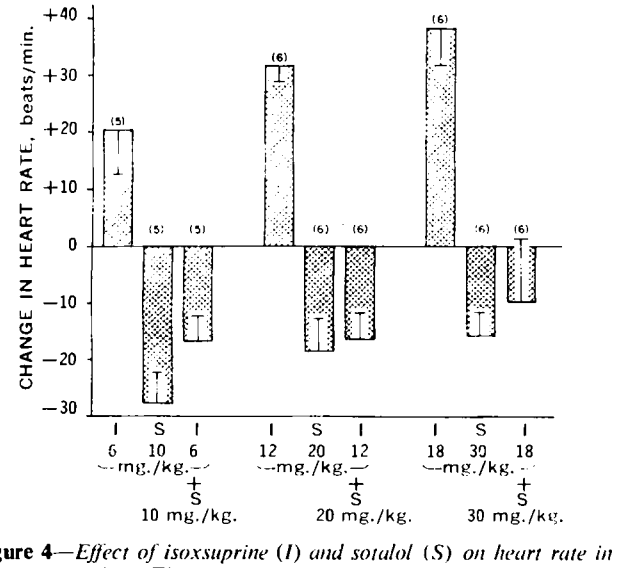

Figure 4—*Effect of isoxsuprine (I) and sotalol (S) on heart rate in hypertensive dogs. The response to sotalol alone is not significantly different from the response to I + S at any dose. See Fig. 3 for details to legend.*

Hydralazine is the only direct acting vasodilating agent currently used in the treatment of hypertension. Despite the capacity of this agent to lower blood pressure, its antihypertensive effectiveness is limited by the reflex increase in sympathetic discharge that produces an undesirable increase in both heart rate and cardiac output. Glick and Braunwald (27) showed that the reflex cardiovascular adjustment initiated by a fall in blood pressure consists of increased sympathetic discharge rather than a decrease in vagal activity. The cardiac augmentation produced by this means may often compromise the depressor response to this agent (12, 13). Several investigators showed that the cardiac augmentation resulting from the hypotensive action of hydralazine can be prevented by the concurrent administration of β-blocking agents (9, 26), but other side effects associated with this agent, *e.g.*, lupus-like reaction, suggest that vasodilating agents such as isoxsuprine and nylidrin would be more valuable adjuncts to β-blockade therapy.

Propranolol is the only β-blocking agent currently available in clinical practice in the United States that can be used in combination with vasodilating drugs. In some respects, sotalol is more desirable than propranolol for this purpose. The use of propranolol with a vasodilating agent may not result in as great a hypotensive response as would result with the use of other β-blockers since it has been reported that propranolol causes an increase in peripheral resistance (16, 28, 29). It is possible, therefore, that the increase in vascular resistance produced by propranolol may directly counteract the decrease in arteriolar resistance produced by the vasodilating drug. There have been no reports to date that sotalol produces such an increase in peripheral vascular resistance. In addition, propranolol has been reported to possess a direct myocardial depressant action (30, 31), which may limit its usefulness in patients with hypertensive heart disease. Sotalol, on the other hand, has been reported to be a "safer" β-blocking agent (32) since it does not directly reduce myocardial function at doses that produce β-blockade (30, 33).

Since at least part of the vasodilation produced by isoxsuprine is the result of stimulation of β-receptors (22) and since propranolol and sotalol block both cardiac and vascular β-receptors, these agents may reduce to some extent the hypotensive effect elicited by isoxsuprine. In this regard, β-blockers, such as practolol, that exert a specificity for blockade of the cardiac β-receptors more so than vascular β-receptors (34) should prove to be even more useful hypotensive drugs when combined with vasodilating agents such as isoxsuprine and nylidrin.

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Detection of Prostaglandin $F_{2\alpha}$ as Pentafluorobenzyl Ester by Electron-Capture GLC

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Abstract □ The pentafluorobenzyl ester of prostaglandin $F_{2\alpha}$ was synthesized on a preparative scale and was gas chromatographed as the tris(trimethylsilyl) ether. The latter was found to be stable during GLC and highly sensitive to electron-capture detection. The lower limit of detection was 12.5 pg. of the ester, injected on-column as the silylated product. The nanogram scale conversion of prostaglandin $F_{2\alpha}$ to the ester, under conditions amenable to electron-capture GLC detection, was developed. The electron-capture GLC response was linear over the 0.03–0.84-ng. range of the ester, injected as the tris(trimethylsilyl) ether.

Keyphrases □ Prostaglandin $F_{2\alpha}$—electron-capture GLC analysis as pentafluorobenzyl ester □ GLC, electron-capture detection—analysis, prostaglandin $F_{2\alpha}$ as pentafluorobenzyl ester

Prostaglandins are biologically important compounds that are active at very low concentrations, usually in the nanogram range. To study the physiological role and the mechanisms of action, as well as the absorption, metabolism, and excretion of these prostaglandins, simplified analytical methodology of high sensitivity and specificity is required. Among the many methods considered for the quantitation of prostaglandins are those based on biological responses (1, 2), enzymatic assay (3), fluorescence and UV spectroscopy (4–6), radioimmunoassay (7–11), and chromatography. The last includes: (*a*) high-pressure liquid chromatography for the detection of low levels of prostaglandins after conversion to the C-1-*p*-nitrobenzyl esters (12), and (*b*) GLC utilizing flame-ionization detection (13–15), mass spectrometric detection (16–22), or electron-capture detection (23–26). GLC methods utilizing flame ionization usually lack adequate sensitivity for the detection of low levels of prostaglandins incident in biological fluids. The GLC–mass spectrometric methods and the electron-capture GLC methods, which afford high sensitivity, also offer a high degree of specificity when combined with a preliminary TLC separation. In terms of general utility, the widely used and elegant GLC–mass spectrometric method has the disadvantage of requiring expensive and usually inaccessible instrumentation, as well as the synthesis of the isotopically labeled (deuterated) prostaglandins for internal standards and carriers.

Reported electron-capture GLC methods based on the inherent electron-capturing properties of prostaglandin B (PGB) compounds (23, 24) have drawn considerable attention. This approach, however, is limited to the PGB compounds or to prostaglandins that can be readily and quantitatively converted to the PGB's. The most common derivatives of the prostaglandins examined for electron-capture detection involve derivatization of the hydroxy groups as, for example, conversion to a heptafluorobutyrate ester (25, 26) or bromomethyl–dimethylsilyl ether (24). The thermal instability of the heptafluorobutyrate derivative, resulting in the formation of multiple GLC peaks, renders it unsuitable for electron-capture GLC analysis (26).

Since all naturally occurring prostaglandins contain a C-1 carboxyl group that has been shown to undergo facile reaction with benzylic halides, the C_1-penta-