PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 145, 1368–1371 (1974)

# Interaction Between Acetylcholine and Sotalol (MJ-1999) in Adrenal Medulla (38015)

ANTHONY G. PROAKIS, JOSEPH L. BOROWITZ, MICKEY R. MCDOUGAL, AND GEORGE R. SPRATTO
(Introduced by T. S. Miya)
*Department of Pharmacology and Toxicology, Purdue University, Lafayette, Indiana 47907*

This work was prompted by the suggestion that the hyperglycemia produced by the *beta* adrenergic blocker, sotalol, is due to adrenal catecholamine release (1). This suggestion was tested directly in isolated bovine adrenals.

*Materials and Methods.* Bovine adrenals were obtained at a local slaughterhouse, placed on ice during transport, and used about 1 hr post mortem. The glands were perfused through the adrenal vein at a rate of 5 ml/min with aerated, tris-buffered, Lockes solution (2) at 24° or 31°C. Catecholamine release was measured colorimetrically (3).

Three concentration-response curves to acetylcholine or potassium were obtained at 15 min intervals to evaluate the effect of sotalol. Sotalol was included in the middle of the 3 concentration-response series. This was done because the response to acetylcholine (4) or potassium decreases on repeated stimulation probably due to depletion of catecholamine stores. Thus, inhibition was indicated when the second concentration-response series (which included sotalol) was below the third or recovery concentration-response series. Enhancement was indicated when catecholamine release in the presence of sotalol was elevated above the initial control level.

All $P$ values given were obtained by comparison of paired differences.

*Results.* Sotalol ($10^{-3}$ $M$, 10 ml) is a weak agonist in isolated bovine adrenals. Only 13 of 44 adrenals so treated increased their output of amines above resting secretion at 31°C, and no change in catecholamine release was detected in the remaining glands. The glands that responded to sotalol increased their amine output an average of about 1 $\mu$g/min. A slight response was also obtained in 2 of 12 glands treated similarly with $10^{-2}$ $M$ sotalol, but no response was noted in 4 glands exposed to $10^{-4}$ $M$ sotalol. A slight response was also noted in 1 of 4 glands infused at 24°C with 10 ml of $10^{-3}$ $M$ sotalol.

Table I shows that $10^{-3}$ $M$ sotalol enhanced the response of adrenals to acetylcholine at 24°C by about 20% ($P < .05$). However, when the same experiments were done at 31°C, an inhibition of 27% was seen ($P < .02$). Thus an inhibitory effect of sotalol is more evident when the temperature is elevated.

Figure 1 shows the effect of sotalol, $10^{-3}$ $M$, on the concentration-response curve to acetylcholine. At the lowest concentration of acetylcholine ($6.7 \times 10^{-5}$ $M$), adrenal catecholamine release is enhanced by sotalol ($P < .05$). The response to the higher concentrations of acetylcholine, however, are depressed by sotalol ($P < .02$ at 2.0 m$M$ acetylcholine).

In contrast to the results with acetylcholine, the concentration-response curve to potassium (Fig. 2) is not affected by $10^{-3}$ $M$ sotalol. The tachyphylaxis of the adrenal response to potassium is no greater in the presence than in the absence of $10^{-3}$ $M$ sotalol. Sotalol, $10^{-2}$ $M$, depressed the response to potassium (Fig. 2) but did not alter the shape of the curve.

Figure 3 shows a depression of acetylcholine-induced adrenal catecholamine re-

TABLE I. Effect of Sotalol on Adrenal Catecholamine Release by Acetylcholine.[a]

| Catecholamine Release, μg/min | | | |
|---|---|---|---|
| 24°C | | 31°C | |
| Mean of control & recovery | Sotalol | Mean of control & recovery | Sotalol |
| 22.5 | 30.6 | 222 | 130 |
| 25.6 | 27.5 | 156 | 129 |
| 43.1 | 65.6 | 130 | 101 |
| 48.1 | 55.6 | 142 | 115 |
| 130.6 | 184.4 | 150 | 101 |
| 122.5 | 121.3 | 123 | 94 |
| 81.3 | 96.9 | 106 | 83 |
| 76.9 | 85 | 109 | 80 |
| 53.1 | 65.6 | | |
| 59.4 | 59.4 | | |
| 19.4 | 30.6 | | |
| 24.4 | 22.5 | | |
| Ave. 58.9 | 70.4 | 142 | 104 |

[a] Glands were exposed to acetylcholine ($6.7 \times 10^{-4} M$, 10 ml) at 24°C or 31°C before, during and after treatment with 10 ml of $10^{-3} M$ sotalol. Results of individual experiments are shown so that the response in the presence of sotalol can be compared to control responses in the same gland. An interval of 15 min was allowed between each stimulus. The differences are significant both at 24°C ($P < .05$) and 31°C ($P < .02$). When these experiments were done without sotalol, no significant differences were seen.

lease by $10^{-2} M$ sotalol. The degree of inhibition is greater than that seen when potassium was used as the stimulus. Also note that the residual effects of sotalol appear to alter the shape of the recovery concentration-response curve to acetylcholine.

*Discussion.* The interaction between the weak agonist sotalol and acetylcholine in

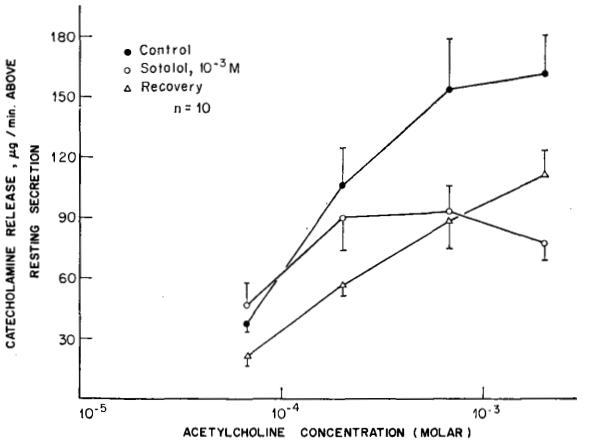


FIG. 1. Effect of $10^{-3} M$ sotalol on the concentration-response curve of adrenal medulla to acetylcholine. The response to $6.7 \times 10^{-5} M$ acetylcholine was significantly enhanced by sotalol ($P < .05$). The response to $2 \times 10^{-3} M$ acetylcholine was significantly less than the corresponding point in the recovery curve ($P < .01$). The experiments were done at 31°C.

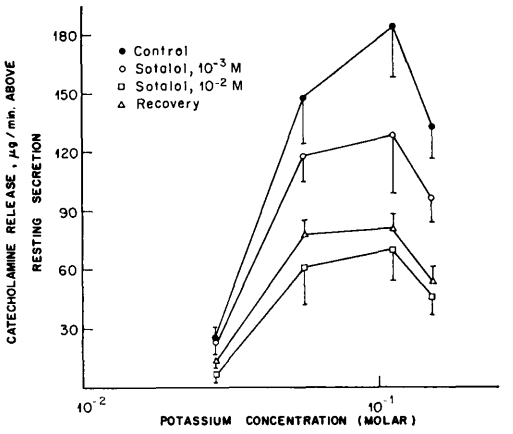


FIG. 2. Effect of sotalol on the concentration-response curve of adrenal medulla to potassium. Potassium concentrations were infused successively at 31°C. Either $10^{-3}$ or $10^{-2}$ *M* sotalol was used in each experiment. Control and recovery curves were combined for the experiments with $10^{-3}$ and $10^{-2}$ *M* sotalol.

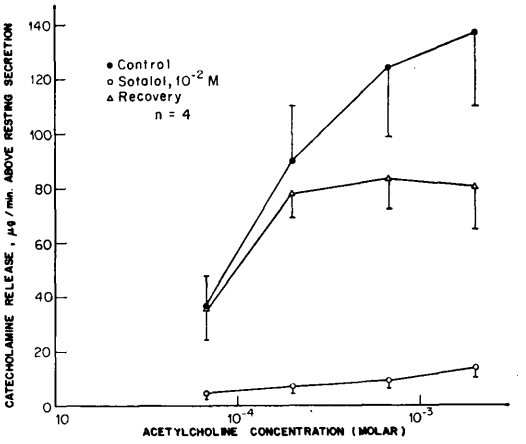


FIG. 3. Effect of $10^{-2}$ *M* sotalol on the concentration-response curve of adrenal medulla to acetylcholine. Conditions were the same as for Fig. 1, except for the concentration of sotalol.

adrenal medulla is similar to the competitive dualism described by Ariens (1964) (5). Accordingly low concentrations of sotalol enhance, and high concentrations of sotalol inhibit the effect of acetylcholine. Thus, a weak agonist effect in adrenal medulla probably explains the hyperglycemic action of sotalol in mice (1).

Sotalol's agonist effect in isolated bovine adrenal medulla was undetectable in many glands under the conditions of these experiments. When catecholamine release was detected it amounted to about 1 μg/min. Yet, sotalol enhanced the action of $6.7 \times 10^{-4}$ *M* acetylcholine by about 10 μg/min. Binding of sotalol to silent cholinergic receptors may explain this potentiation of acetylcholine.

Sotalol is known to have weak local anesthetic properties (6), and may be similar to other local anesthetics (procaine, dimethyl-procaine, and amethocaine) which have weak agonist effects in adrenal medulla (7). It has been reported that procaine, dimethyl-procaine and amethocaine interact with the acetylcholine receptor in adrenal medulla since their action is blocked by atropine and hexamethonium (7).

Sotalol, $10^{-2}$ *M*, inhibited adrenal catecholamine release by acetylcholine better than potassium induced release. This indicates a preferential block of the cholinergic receptors in adrenal medulla relative to the receptors mediating the potassium induced response. Sotalol resembles procaine in this regard since procaine is more effective against acetylcholine than against a high potassium medium to which calcium is added (7). By contrast propranolol, a strong local anesthetic, is equally effective against acetylcholine or against calcium in a high potassium medium. It appears that propranolol exerts only a local anesthetic effect which blocks both types of stimuli equally well. Sotalol and procaine apparently have ganglionic blocking effects in addition to their local anesthetic actions.

Ganglionic effects of sotalol may be responsible not only for the hyperglycemia but also in part for the antianxiety actions of this agent especially when it is used in high doses (e.g., 800 mg/day in the human) (8).

*Summary.* Sotalol (MJ-1999) appears to act as a weak agonist in releasing catecholamines from isolated perfused bovine adrenals. Low concentrations of sotalol enhance and high concentrations inhibit acetylcholine-induced adrenal catecholamine release. Adrenal catecholamine release by high potassium media is decreased by

sotalol only at high concentrations. Thus ganglionic actions of sotalol are seen at lower concentrations than are the local anesthetic actions. This study supports the suggestion that the effect of sotalol on blood sugar is due to enhanced adrenal catecholamine release and suggests that ganglionic blockade can be produced by sotalol in concentrations below those required for local anesthetic action.

This work was supported by NIH Grant #AM 16153.

1. Born, C. K., Spratto, G. R., Fed. Proc. 30, 315 Abs (1971).

2. Borowitz, J. L., Am. J. Physiol. 220, 1194 (1971).

3. von Euler, U. S., and Hamberg, U., Acta Phys. Scand. 19, 74 (1949).

4. Rubin, R. P., and Jaanus, S. D., Arch. Pharmakol. Exp. Pathol. 256, 464 (1967).

5. Ariens, E. J., Simonis, A. M., and Van Rossum, J. M., *in* "Molecular Pharmacology" (E. J. Ariens, ed.), p. 169. Academic Press, New York.

6. Davis, W. G., J. Pharm. Pharmacol. 22, 284 (1970).

7. Jaanus, S. D., Miele, E., and Rubin, R. P., Brit. J. Pharmacol. 31, 319 (1967).

8. Tyrer, P. J., and Lader, M. H., Clin. Pharmacol. & Therap. 14, 418 (1973).

Received Sept. 17, 1973. P.S.E.B.M., 1974, Vol. 145.