REFERENCES

BLUMBERG, A., DENNY, S., NISHIKAWA, K., PURGE, E., MARSHALL, G. R. & NEEDLEMAN, P. (1976). *Prostaglandins*, **11**, 195–197.

CHAND, N. & EYRE, P. (1977). *J. Pharm. Pharmac.*, **29**, 387–388.

ERCAN, Z. S. & TÜRKER, R. K. (1977). *Agents and Actions*, **7**, 569–572.

TÜRKER, R. K. & KHAIRALLAH, P. A. (1969). *J. Pharm. Pharmac.*, **21**, 498–501.

TÜRKER, R. K. & ERCAN, Z. S. (1976). *Ibid.*, **28**, 291–301.

VANE, J. R. (1971). *Nature, New Biol.*, **231**, 232–235.

Effect of labetalol and guanethidine on contractile responses to acetylcholine in the rat anococcygeus muscle

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Labetalol is a competitive antagonist at α- and β-adrenoceptors (Brittain & Levy, 1976). It is 6–10 times less potent than phentolamine in blocking α-adrenoceptors, 1·5–3 times less potent than propranolol in blocking β-adrenoceptors, and, hence, 4–8 times more potent at β- than at α-adrenoceptors. This profile of labetalol is unique and has provided an antihypertensive agent which has been successfully used in clinical trials (Prichard & Boakes, 1976). We have investigated the effects of labetalol on contractile responses to acetylcholine in the rat anococcygeus muscle, a tissue which has no cholinergic innervation (Gillespie, 1972).

Mature male Wistar rats killed by a sharp blow at the base of the skull were exsanguinated. Anococcygeus muscles were dissected as described by Gillespie (1972) and mounted under 0·5 g tension in 5 ml organ baths containing a modified Krebs solution at 37°, equilibrated with 5% CO_2 in oxygen. Isometric contractions were recorded with force displacement transducers (Grass, FT03.C) connected to a polygraph (Grass model RPS 7C8A).

When the effects of labetalol, guanethidine, or phentolamine on responses were being examined, these drugs were present in the Krebs solution throughout. For the 6-hydroxydopamine experiments, the isolated muscles were incubated in the presence of 10^{-3} M 6-hydroxydopamine for 3 h and then washed in Krebs solution for 30 min. After this treatment, the accumulation of noradrenaline was inhibited, contractile responses to it were potentiated and responses to tyramine were abolished (Doggrell & Woodruff, unpublished observations).

Responses were calculated as a percentage of the maximum response. Each mean value was determined from at least four separate preparations and is expressed ±s.e.m. The responses, under different conditions, were compared by Student's unpaired *t*-test and considered significantly different whenever $P < 0.05$.

The drugs used were labetalol hydrochloride* (Allen & Hanburys), acetylcholine chloride (BDH Canada, Ltd), phentolamine mesylate* and guanethidine sulphate* (Ciba), and 6-hydroxydopamine hydrobromide (Regis Chemicals Ltd). Compounds indicated with an asterisk were generously donated by the companies.

The modified Krebs solution had the following com-

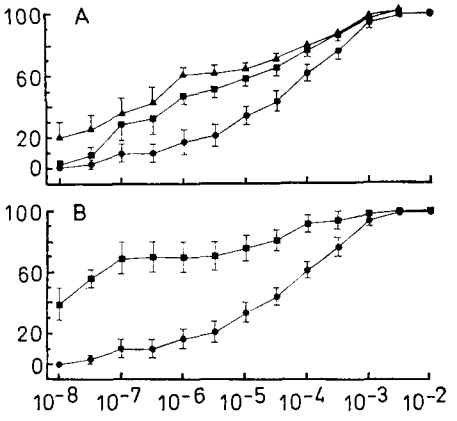

FIG. 1. The effects of labetalol and guanethidine on contractile responses to acetylcholine in the rat anococcygeus muscle. Responses to acetylcholine in normal Krebs solution (●—●). A. Responses to acetylcholine in the presence of 10^{-5} M labetalol (▲—▲) and in the presence of 10^{-6} M labetalol (■—■). B. Responses to acetylcholine in the presence of 6×10^{-6} M guanethidine (■—■). All responses are expressed as a percentage of the maximum response (ordinate). Each value is the mean ±s.e.m. from a minimum of 4 preparations. Abscissa: acetylcholine (M).

* Correspondence.

position (mM): NaCl, 116; KCl, 5·4; $CaCl_2$, 2·5; $MgCl_2$, 1·2; NaH_2PO_4, 1·2; $NaHCO_3$, 22·0, D-glucose, 11·2; Na_2EDTA, 0·04.

Labetalol and guanethidine induce spontaneous activity of the rat anococcygeous muscle which lasts 2–3 h (Doggrell & Paton, 1978). Thus, when studying the effects of these drugs on contractile response to acetylcholine, the tissues were equilibrated until the spontaneous activity disappeared.

10^{-5} and 10^{-6} M labetalol potentiated responses to 10^{-8} – 10^{-4} M and 10^{-7} – 10^{-4} M acetylcholine, respectively, but had no effect in the presence of acetylcholine, 3×10^{-4} – 10^{-2} M (Fig. 1A). 10^{-7} M labetalol had no effect on responses to acetylcholine. Guanethidine (6×10^{-6} M) potentiated responses to acetylcholine 10^{-8} – 3×10^{-4} M but not to 10^{-3} – 10^{-2} M (Fig. 1B). Thus, the maximum response to acetylcholine occurred with a concentration of 3×10^{-3} M in normal Krebs solution and in the presence of labetalol (10^{-5} or 10^{-6} M) or guanethidine (6×10^{-6} M).

The spontaneous activity observed with labetalol (10^{-5} or 10^{-6} M) or guanethidine (6×10^{-6} M) was abolished with phentolamine (5×10^{-6} M) or incubation of the muscle after with 6-hydroxydopamine (10^{-3} M for 3 h); for results with 10^{-5} M labetalol see Fig. 2. Neither phentolamine nor 6-hydroxydopamine had any effect on responses to acetylcholine. In the presence of phentolamine or after 6-hydroxydopamine incubation, labetalol (10^{-5} or 10^{-6} M) and guanethidine (6×10^{-6} M) had no effect on responses to acetylcholine.

Labetalol and guanethidine induce spontaneous activity of the rat anococcygeus muscle (Doggrell & Paton, 1978). Gillespie (1972) demonstrated that guanethidine raised the tone of this preparation by releasing noradrenaline. The spontaneous activity observed with labetalol or guanethidine was abolished in the presence of phentolamine, or after 6-hydroxydopamine. These results suggest that the spontaneous activity observed in the presence of labetalol or guanethidine is due to the release of noradrenaline.

Spontaneous activity with labetalol has not been reported in other tissues i.e., atrial strips, intact tracheal tube and mesenteric vein of guinea-pig (Farmer, Kennedy & others, 1972), rabbit and rat aortic strips (Brittain & Levy, 1976), and cat spleen strips (Blakeley & Summers, 1977). The unexpected ability of labetalol to release noradrenaline in the rat anococcygeus muscle, but not in other tissues, may be due to the dense noradrenergic innervation of this preparation compared with the tissues studied by others.

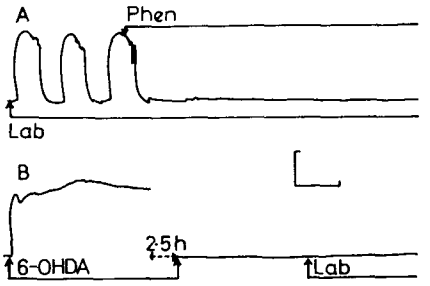

FIG. 2. The effects of phentolamine and 6-hydroxydopamine on the spontaneous activity observed with labetalol in the rat anococcygeus muscle. A. The spontaneous activity observed with 10^{-5} M labetalol was abolished by 5×10^{-6} M phentolamine. B. Following 6-hydroxydopamine incubation (10^{-3} M for 3 h) no spontaneous activity was observed with 10^{-5} M labetalol. Phen: phentolamine; Lab: labetalol; 6-OHDA: 6-hydroxydopamine. Vertical scale: 2 g; horizontal scale: 10 min.

When the spontaneous activity observed with labetalol (10^{-5} or 10^{-6} M) or guanethidine (6×10^{-6} M) had disappeared, these drugs potentiated responses to low concentrations of acetylcholine. This potentiation was abolished by phentolamine or after 6-hydroxydopamine incubation neither of which alone had any effect on responses to acetylcholine. 6-Hydroxydopamine incubation depletes noradrenaline stores (Wadsworth, 1973) and inhibits responses to the indirectly acting tyramine (Gillespie & McGrath, 1975). Thus, these results suggest that the potentiation of responses to acetylcholine by labetalol and guanethidine is due to the release of subthreshold concentrations of noradrenaline.

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REFERENCES

BLAKELEY, A. G. M. & SUMMERS, R. J. (1977). *Br. J. Pharmac.*, **59**, 643–650.
BRITTAIN, G. & LEVY, G. P. (1976). *Br. J. clin. Pharmac.*, **3**, 681–694S.
DOGGRELL, S. & PATON, D. M. (1978). *Br. J. Pharmac.*, **62**, 380 P.
FARMER, J. B., KENNEDY, I., LEVY, G. P. & MARSHALL, R. J. (1972). *Ibid.*, **45**, 660–675.
GILLESPIE, J. S. (1972). *Ibid.*, **45**, 404–416.
GILLESPIE, J. S. & MCGRATH, J. C. (1975). *Ibid.*, **54**, 481–488.
PRICHARD, B. N. C. & BOAKES, A. J. (1976). *Br. J. clin. Pharmac.*, **3**, 743–750S.
WADSWORTH, R. M. (1973). *Eur. J. Pharmac.*, **21**, 383–387.