Some Observations on the Adrenergic Blocking Activity of Desipramine and Amitriptyline on Aortic Strips of Rabbits

Antidepressants: desipramine and amitriptyline are known to potentiate the pressor effects of norepinephrine in vivo[1,2]. It is assumed that this potentiation is due to the inhibition of norepinephrine uptake into the tissue storage sites by antidepressants[3–6], and the resulting increase in the concentration of norepinephrine at the receptor sites. If so, antidepressants can be expected to also potentiate norepinephrine on isolated vascular preparations. Desipramine, however, is known to reduce norepinephrine-induced contractions of aortic strips of rabbits pretreated with reserpine[7]. Since the nature of interaction of antidepressants with norepinephrine at the sympathetic nerve endings may be similar to that in the central nervous system, we became interested in the mechanism of norepinephrine blockade by antidepressants, and found that desipramine antagonizes norepinephrine on aortic strips of rabbits even without reserpine pretreatment, and that amitriptyline is more potent than desipramine as norepinephrine antagonist.

Rabbit aortic strips were isolated in accordance with the technique of FURCHGOTT and BHADRAKOM[8]; 54 preparations from 27 male rabbits of 1–2 kg body weight were used in this study. Aortic strips were suspended in a 10 ml bath with Krebs-Henseleit solution[9] maintained at 37 °C and continuously aerated with 95% O_2 and 5% CO_2 mixture. Initial tension was 2 g. After a $1^1/_2$–2 h equilibration period, norepinephrine (Levophed®) was added to the bath, usually at 5×10^{-8} g/ml concentration. The responses to norepinephrine were recorded on Sanborn Dual Channel, Model 321, carrier amplifier-recorder through a Sanborn, Model FTA-3-1, force transducer. Norepinephrine was left in the bath until the maximal response was clearly reached, usually for 8–10 min. The bath was washed 3 times with fresh Krebs-Henseleit solution. Antagonists were added to the bath 10 min prior to norepinephrine. The decrease of the subsequent response to norepinephrine was used as a measure of the effect of antagonists. The activity was expressed in percent reduction of the control response to norepinephrine.

Amitriptyline and desipramine reduced norepinephrine-induced contractions of rabbit aortic strips (Figure 1). The effects of both antagonists persisted for over 1 h in spite of repeated exchanges of the bathing medium. Even after 1 h higher concentrations of norepinephrine were required to restore the contractile response to the control level. The cumulative dose-response curve for norepinephrine was shifted to the right and the maximal response to norepinephrine was slightly reduced by desipramine, 1×10^{-7} g/ml (Figure 2). If administered during sustained norepinephrine-induced contraction, desipramine had a relaxant effect at 5×10^{-5} but not at 5×10^{-7} g/ml. Phentolamine at 5×10^{-7} g/ml relaxed aortic strips contracted with norepinephrine.

Chlorpromazine, which is known to antagonize norepinephrine-induced contractions of rabbit aortic strips[10] was also effective under our experimental conditions. At 5×10^{-9} g/ml chlorpromazine reduced norepinephrine-induced contractions by 18% and at 5×10^{-8} g/ml by 59% (average values for 11 and 14 experiments, respectively). These results indicated that chlorpromazine is considerably more potent than desipramine and slightly more potent than amitriptyline as norepinephrine antagonist on rabbit aortic strips.

There appears to be no correlation between the antidepressant activity of drugs and their ability to antagonize norepinephrine on rabbit aortic strips. It is possible, however, that norepinephrine antagonism can be correlated with the tranquilizer component of action of the antidepressant drugs. Chlorpromazine is more potent

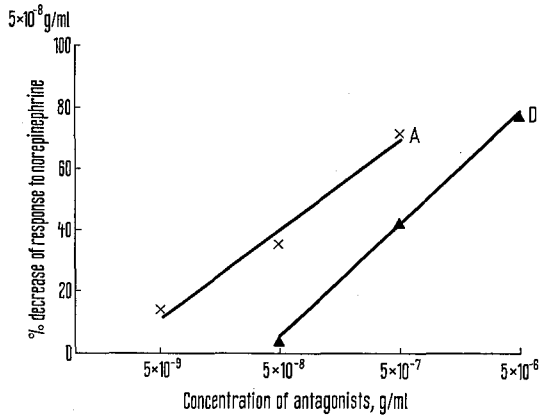

Fig. 1. Dose-response regression lines for antagonism of norepinephrine (5×10^{-8} g/ml) – induced contractions of rabbit aortic strips by amitriptyline (A) and desipramine (D). The lines were calculated on the basis of 21–25 observations for each antagonist.

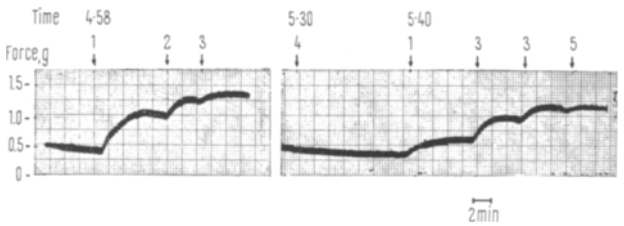

Fig. 2. Aortic strips from rabbit, male, 2.0 kg. Cumulative dose-response curve for norepinephrine before and after addition of desipramine. At 1, norepinephrine, 5×10^{-8} g/ml; at 2, norepinephrine, total 3×10^{-7} g/ml; at 3, norepinephrine, total 1×10^{-6} g/ml; at 4, desipramine, 5×10^{-7} g/ml; at 5, norepinephrine, total 1.3×10^{-6} g/ml. Note that dose-response curve for norepinephrine was shifted to the right and the maximal effect was slightly reduced by desipramine.

[1] A. KAUMANN, N. BASSO and P. ARAMENDÍA, J. Pharmac. exp. Ther. *147*, 54 (1966).
[2] H. BESENDORF, F. A. STEINER and A. HÜRLIMANN, Schweiz. Med. Wschr. *92*, 244 (1962).
[3] H. J. DENGLER, H. E. SPIEGEL and E. O. TITUS, Nature *191*, 816 (1961).
[4] L. L. IVERSEN, *The Uptake and Storage of Noradrenaline in Sympathetic Nerves* (Cambridge University Press 1967).
[5] L. L. IVERSEN, Adv. Drug Res. *2*, 1 (1965).
[6] J. GLOWINSKI and J. AXELROD, Nature *204*, 1318 (1964).
[7] R. K. TÜRKER and P. A. KHAIRALLAH, Experientia *23*, 252 (1967).
[8] R. F. FURCHGOTT and S. BHADRAKOM, J. Pharmac. exp. Ther. *108*, 129 (1953).
[9] H. A. KREBS and K. HENSELEIT, Hoppe-Seyler's Z. physiol. Chem. *210*, 33 (1932).
[10] S. D. GOKHALE, O. D. GULATI and H. M. PARIKH, Br. J. Pharmac. *23*, 508 (1964).

than either amitriptyline or desipramine as an antagonist of norepinephrine-induced contractions. Amitriptyline, which is known to have a substantial tranquilizer component of action, is more potent than desipramine. Antagonism of norepinephrine-induced contractions on rabbit aortic strips may, therefore, be of value as an indirect measure of potential tranquilizer component of action of new antidepressant drugs.

Of particular interest was our observation that in 5 out of 54 aortic strip preparations, norepinephrine, 5×10^{-8} g/ml, caused relaxation. At a higher concentration, norepinephrine had a greater relaxant effect. In the second aortic strip from the same animal norepinephrine caused the usual contraction. Propranolol, 5×10^{-6} or 5×10^{-5} g/ml, blocked norepinephrine-induced relaxation of aortic strips in 2 out of 2 preparations. This blockade was overcome with a higher dose of norepinephrine (Figure 3). In one experiment, desipramine, 5×10^{-6} g/ml, and in another, amitriptyline, 5×10^{-7} g/ml, blocked the relaxant effect of norepinephrine (Figure 4). In one additional preparation, norepinephrine, 5×10^{-7} g/ml, caused a biphasic response: a contraction followed by a relaxation. Both phases of this response were reduced by desipramine, 5×10^{-7} g/ml.

It is possible that amitriptyline and desipramine compete for α- as well as β-adrenergic receptors with norepinephrine. Other possibilities also exist. In aortic strip preparations norepinephrine may have to be transported through one or more membranes before it reaches either α- or β-adrenergic receptors. Desipramine and amitriptyline may block the transport of norepinephrine through these membranes and, therefore, reduce the concentration of norepinephrine at the receptors of both types.

Response to norepinephrine can also be blocked at a level or levels distal to adrenergic receptors as suggested for diazoxide by SCRIABINE and BOOHER[11] and later demonstrated by WOHL et al.[12,13]. There are probably numerous sites distal to the adrenergic receptors at which an effect, initially caused by norepinephrine, can be blocked. The norepinephrine-induced contraction of aortic strips is likely to involve an increase in free myoplasmic Ca^{++}. This can be achieved by increasing Ca^{++} influx, increasing release of Ca^{++} from intracellular sources, i.e. sarcoplasmic reticulum, or by depressing calcium uptake. Chlorpromazine and antidepressants may interfere with any of the processes responsible for the increase in free myoplasmic Ca^{++}, or as postulated for diazoxide[12], these drugs may compete with Ca^{++} for the hypothetical Ca^{++} receptors. Desipramine was recently reported to block the contractile effect of Ca^{++} on isolated 'depolarized' rat mesenteric artery without reducing the entry of Ca^{++} into the muscle fiber[14]. The sarcoplasmic Ca^{++} pump in rabbit skeletal muscle was shown to be inhibited by chlorpromazine and imipramine[15,16]. It is, therefore, conceivable that antidepressants may reduce release of Ca^{++} from as well as the uptake by the sarcoplasmic reticulum. Since relaxation of aortic strips in response to norepinephrine, observed in our experiments, may have been associated with an increase in uptake of Ca^{++} by the reticulum, inhibition of intracellular Ca^{++} transport may be expected to block norepinephrine-induced relaxation as well as contraction[17].

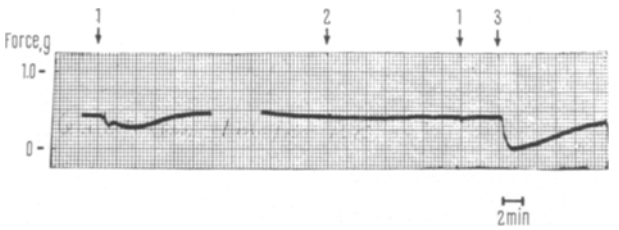

Fig. 3. Antagonism of norepinephrine-induced relaxation of aortic strips by propranolol. Rabbit, male, 2 kg. At 1, norepinephrine, 5×10^{-8} g/ml; at 2, propranolol, 5×10^{-5} g/ml; at 3, norepinephrine, 5×10^{-7} g/ml.

Zusammenfassung. Desipramin und Amitriptylin hemmen die durch Noradrenalin hervorgerufenen Kontraktionen der isolierten Aortenstreifen der Kaninchen. Es wird angenommen, dass die Hemmung des Noradrenalins durch Antidepressiva nicht auf einer Hemmung der α-Rezeptoren beruht.

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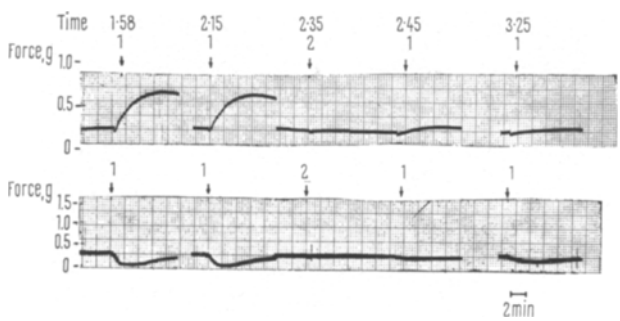

Fig. 4. Antagonism of norepinephrine-induced contraction and relaxation of aortic strips by amitriptyline, 5×10^{-7} g/ml. Rabbit, female, 1.4 kg. Both strips are from the same animal. At 1, norepinephrine, 5×10^{-8} g/ml; at 2, amitriptyline, 5×10^{-7} g/ml. Note the blockade of both types of responses to norepinephrine by amitriptyline.

[11] A. SCRIABINE and K. D. BOOHER, Pharmacologist *6*, 166 (1964).
[12] A. J. WOHL, L. M. HAUSLER and F. E. ROTH, J. Pharmac. exp. Ther. *158*, 531 (1967).
[13] A. J. WOHL, L. M. HAUSLER and F. E. ROTH, Life Sci. *7*, 381 (1968).
[14] B. J. NORTHOVER, Br. J. Pharmac. *32*, 426P (1968).
[15] H. BALZER, M. MAKINOSE and W. HASSELBACH, Arch. exp. Path. Pharmak. *260*, 444 (1968).
[16] H. BALZER, M. MAKINOSE, W. FIEHN and W. HASSELBACH, Arch. exp. Path. Pharmak. *260*, 456 (1968).
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