It is remarkable that despite the great difference of H reactivity between control and adapted animals there is no such difference in histaminase activities. This suggests that the enzymatic process does not play a basic role in the development of smooth muscle adaptation to histamine[10].

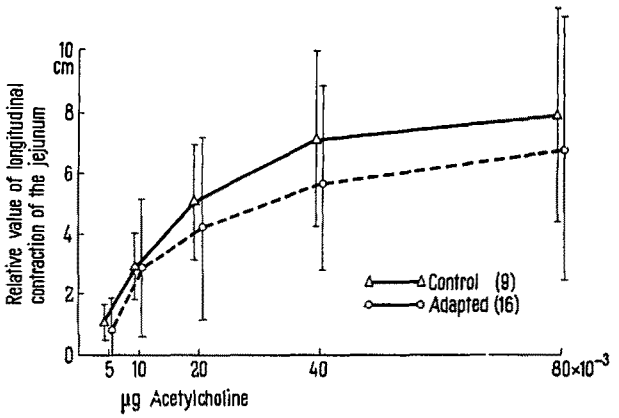

Fig. 2. The points on the curves represent mean value with the standard deviation of the ileum contraction in cm after administration of acetylcholine. The corresponding points on these two curves show no significant difference. In parentheses number of guinea-pigs used in the experiment are shown.

Résumé. L'adaptation des cobayes à l'histamine par voie intrapéritonéale diminue la sensibilité de leur jéjunum isolé à l'histamine. En même temps le jéjunum isolé de cobayes adaptés réagit à l'acétylcholine sans changer remarquablement de susceptibilité. L'activité de l'histaminase dans le jéjunum adapté ne démontre pas la différence en relation avec celle du jéjunum non adapté.

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[10] We thank Hoffmann-La Roche Ltd. for kindly supplying Histamine dihydrochlorid.

Dual Site of Action of Phenoxybenzamine in the Cat's Spleen: Blockade of α-Adrenergic Receptors and Inhibition of Re-uptake of Neurally Released Norepinephrine

Whether the sites primarily involved in the removal of exogenous and endogenous norepinephrine in a sympathetically innervated organ like the cat's spleen are the receptors of the effector organ, as proposed by BROWN and GILLESPIE[1,2] or rather sympathetic nerve endings, as advanced by PATON[3], is still a matter of controversy. We recently reported[4] that in the isolated perfused spleen of the cat, when removal of infused norepinephrine had been almost completely prevented by cocaine, blockade of smooth muscle receptors by phenoxybenzamine failed to increase further the amount of norepinephrine appearing in the venous effluent. This was taken as evidence that combination of norepinephrine with receptors of the effector organ was of only minor importance for the inactivation of exogenous norepinephrine. It might be objected that the mechanisms of inactivation are not identical for infused and neurally released norepinephrine. The results of the present study, however, lead us to believe that our previous conclusions may be applied to neurally released norepinephrine as well.

Isolation and perfusion of the spleen was performed as described in detail by THOENEN et al.[5]. The splenic nerves were stimulated at 8 min intervals for 10 sec at a rate of 6/sec, with monophasic rectangular pulses of 1 msec duration. Supramaximal voltage was used. Volume changes were recorded with a piston recorder and perfusion pressure with a Condon type Hg-manometer, the pressure changes being a measure of vascular resistance, since perfusion rate (7.5 ml/min) was kept constant. At the onset of every stimulation period the venous effluent was collected in chilled graduated centrifuge tubes for 90 sec. After this time the noradrenaline concentration had returned to levels below the sensitivity of the assay method in untreated preparations. Under phenoxybenzamine infusion the return to prestimulation levels was delayed and the collecting period was prolonged to 150 sec. The norepinephrine content of the venous effluent was assayed on the blood pressure of pithed rats.

After several stimulation periods with constant effect on volume and vascular resistance an infusion of 3 µg/min followed by 10 µg/min of phenoxybenzamine was started. The concentrations perfusing the spleen thus amounted to 0.4 and 1.3 µg/ml. The addition of corresponding amounts of phenoxybenzamine to norepinephrine test solutions had no influence on the norepinephrine assay.

The extent and time course of the adrenergic blocking effect and of the effect on the norepinephrine output during phenoxybenzamine infusion are shown in a representative experiment in Figure 1. In Figure 2 the results of 8 experiments are summarized. For better comparison relative rather than absolute terms were used, since in the control periods the changes in volume and vascular resistance as well as the norepinephrine output varied considerably from one experiment to another, whereas these values were remarkably constant in the same preparation. The contraction area (area enclosed by the lever's writing point from the beginning of the stimulation to its return to the starting level) was chosen as a representative measure of the mechanical response including

[1] G. L. BROWN and J. S. GILLESPIE, J. Physiol. *138*, 81 (1957).
[2] G. L. BROWN, *Adrenergic Mechanisms*. Ciba Foundation Symposium (J. A. Churchill, London 1960), p. 116.
[3] W. D. M. PATON, *Adrenergic Mechanisms*. Ciba Foundation Symposium (J. A. Churchill, London 1960), p. 124.
[4] H. THOENEN, A. HÜRLIMANN, and W. HAEFELY, Exper. *19*, 601 (1963).
[5] H. THOENEN, A. HÜRLIMANN, and W. HAEFELY, Helv. physiol. pharmacol. Acta *21*, 17 (1963).

both intensity and duration of splenic volume changes. The mean of two control values (norepinephrine output and contraction area) was taken as 100% and the subsequent values were expressed as percentual changes.

(a) *The adrenergic blocking effect* of phenoxybenzamine was already maximal at the first stimulation period after starting the infusion of 3 μg/min. At the subsequent stimulation periods during the infusion of 3 and then 10 μg/min no further increase of the blocking action occurred (Figures 1, 2). On the contrary, the response to sympathetic nerve stimulation, i.e. the changes in volume and vascular resistance were partially restored. In the mean of 8 experiments (Figure 2) the contraction area was $10.3 \pm 1.4\%$ of the controls (100%) at the first stimulation period after starting the phenoxybenzamine infusion of 3 μg/min. In the subsequent periods significantly smaller blocking effects were observed ($P < 0.005$).

(b) *The augmentation of the norepinephrine output* on the other hand was relatively small at the first stimulation period after starting the phenoxybenzamine infusion (Figures 1, 2), but increased progressively in the following stimulation periods, finally reaching values 4 times higher than in the control periods (Figure 2).

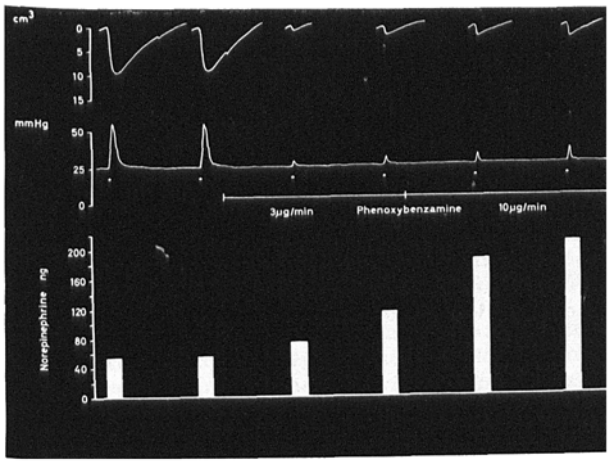

Fig. 1. The effect of phenoxybenzamine on the response to postganglionic stimulation of the isolated perfused spleen of the cat. Simultaneous determination of norepinephrine output (ng), changes in volume (cm³) and vascular resistance (mmHg).

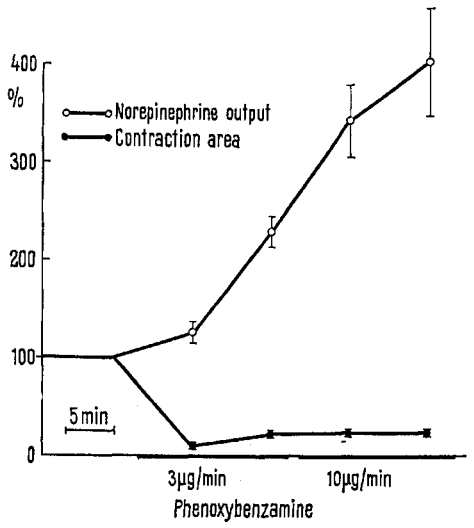

Fig. 2. Comparison between percentual (controls 100%) changes of norepinephrine output and splenic contraction during phenoxybenzamine-infusions of 3 and 10 μg/min.

Our failure to find a correlation between the time course and the intensity of adrenergic blockade, and the increase in norepinephrine output under the influence of phenoxybenzamine, is in our opinion strong evidence against the view of BROWN[2] and of KIRPEKAR and CERVONI[6] that the increased norepinephrine output after phenoxybenzamine is due to blockade of α-adrenergic receptors. The results of the present study are in agreement with those of our previous experiments with norepinephrine infusions[5]. We interpret them as demonstrating a dual site of action of phenoxybenzamine, one at the α-adrenergic receptors of splenic smooth muscles, the other at the norepinephrine storage sites. The former is responsible for the adrenergic blocking action of phenoxybenzamine, the latter for the increased norepinephrine output. These two effects can be differentiated. The blocking effect on splenic contraction occurs rapidly but is subsequently overcome in part by the more slowly appearing but progressively increasing blockade of norepinephrine uptake, which leads to an increase in the amount of free norepinephrine available to the smooth muscle receptors.

An inhibition of norepinephrine uptake by phenoxybenzamine has also been demonstrated by DENGLER et al.[7] and HERTTING et al.[8] in the heart. This block leads to augmented norepinephrine concentrations in the biophase, and thereby to increased responses of the heart to exogenous[9] and endogenous[10] norepinephrine, since phenoxybenzamine has no blocking action on the cardiac β-adrenergic receptors.

In the spleen, however, phenoxybenzamine blocks the α-adrenergic smooth muscle receptors and the resulting inhibition of contraction is only partly overcome by the blocking effect of phenoxybenzamine at norepinephrine uptake sites.

Thus the specific receptor sites for norepinephrine show greater selectivity in their reaction with drugs than the sites of norepinephrine uptake.

Zusammenfassung. An der isoliert durchströmten Milz der Katze wird durch Phenoxybenzamin die bei elektrischer Reizung der Milznerven im venösen Ausfluss erscheinende Noradrenalinmenge vermehrt. Es besteht keine zeitliche und quantitative Korrelation zwischen dieser Noradrenalinvermehrung und dem Ausmass der adrenergen Blockierung. Phenoxybenzamin scheint die Vermehrung des Noradrenalins im venösen Ausfluss durch eine Blockierung der Wiederaufnahme in die Speicher und nicht durch die Blockierung der adrenergen Rezeptoren des Erfolgsorgans hervorzurufen.

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Abteilung für experimentelle Medizin der F. Hoffmann-La Roche & Cie., AG., Basel (Switzerland),
March 5, 1964.

[6] S. M. KIRPEKAR and P. CERVONI, J. Pharmacol. *142*, 59 (1963).
[7] H. J. DENGLER, H. E. SPIEGEL, and E. O. TITUS, Nature *191*, 816 (1961).
[8] G. HERTTING, J. AXELROD, and L. G. WHITBY, J. Pharmacol. *134*, 146 (1961).
[9] A. STAFFORD, Brit. J. Pharmacol. *21*, 361 (1963).
[10] S. HUKOVIĆ, Brit. J. Pharmacol. *14*, 372 (1959).