Organon S.A.,   B. B. VARGAFTIG
Pharmacological Research and Development Department,   N. DAO HAI
60 Eragny sur Epte, France.

October 27, 1971

REFERENCES

ANGGARD, E. & SAMUELSSON, B. (1965). *J. biol. Chem.*, **210**, 3518–3521.
GRYGLEWSKI, R. & VANE, J. R. (1971). *Br. J. Pharmac.*, **43**, 420P–421P.
KUNZE, J. & VOGT, W. (1971). In *Annals of the N.Y. Academy of Sciences Prostaglandins*, pp. 123–125. Editor: Ramwell, P. & Shaw, J. E. New York.
LANDS, W., LEE, R. & SMITH, W. (1971). *Ibid.*, pp. 107–122.
MARKUS, H. B. & BALL, E. G. (1969). *Biochim. biophys. Acta*, **187**, 486–491.
PIPER, P. J. & VANE, J. R. (1969). *Nature, Lond.*, **223**, 29–35.
VARGAFTIG, B. B. & COIRON, M. (1971). *J. Pharmac., Paris*, **2**, 155–173.
VARGAFTIG, B. B. & DAO HAI, N. (1971). *Pharmacology*, **6**, 99–108.
VARGAFTIG, B. B. & DAO HAI, N. (1972). *Europ. J. Pharmac.* In the press.
WILLIS, A. L. (1970). *Pharmac. Res. Commun.*, **2**, 297–304.

Effects of phenoxybenzamine on transmitter release and effector response in the isolated portal vein

The output of noradrenaline from adrenergically innervated tissues is much increased at sympathetic stimulation after α-adrenoceptor blocking agents, e.g., phenoxybenzamine (PBZ) (Brown & Gillespie, 1957; Thoenen, Hürlimann & Haefely, 1964; Boullin, Costa & Brodie, 1967) and such drugs have often been used in the quantitative measurement of transmitter release (see e.g., Boullin & others, 1967; Stjärne, Hedqvist & Bygdeman, 1969; Langer & Vogt, 1971). The increased output of noradrenaline after PBZ has been ascribed to different actions of the drug, especially to blockade of the neuronal reuptake of noradrenaline and to α-adrenoceptor blockade, but there is no general consensus about the relative importance of the different mechanisms. We considered it of interest to study simultaneously the release of [³H]noradrenaline (³H-NA) and the degree of α-adrenoceptor blockade during nerve stimulation at graded concentrations of PBZ. The study was made *in vitro* on a thin tissue, the isolated rat portal vein, in which the conditions for transmitter diffusion are good. The release of noradrenaline was studied with the use of ³H-NA (Häggendal, Johansson & others, 1970).

The basic procedure was that of Häggendal & others (1970). Three isolated portal veins from rats of the Sprague-Dawley strain were mounted in parallel and isometric recording of the mechanical activity was made from one of these vessels. After an accommodation period of 1 h in an organ bath containing a modified Krebs solution (for details see Häggendal & others, 1970) 1-³H-NA was added (10^{-7}M, specific activity 2·34 Ci/mmol, Radiochemical Centre, Amersham, England). After 30 min of incubation another organ bath (volume 1·5 ml) was placed in position and the portal veins were continuously superfused at a rate of 1 ml/min with Krebs solution. The mechanical activity was recorded continuously and the superfusate was sampled throughout for measurement of total radioactivity. During the first

30 min of washout six superfusate samples were collected during 5 min periods. Electrical field stimulation of the intramural adrenergic nerves (4 Hz, 0·8 ms, 15 V) was then performed for 2 min, during which the superfusate was collected. After stimulation, three 5 min washout periods followed before the next stimulation began. Three such 2 min periods of nerve activity with subsequent 15 min washout phases were included in each experiment. PBZ (Dibenzyline; Smith, Kline & French) was added to the Krebs solution immediately after the first stimulation period in six out of eight experiments undertaken.

At the end of the experiments the preparations were blotted between filter paper, weighed, homogenized in 2 ml 2N HCl by an Ultra-Turrax homogenizer before the tissue extracts were centrifuged and filtered. The superfusates were acidified and 15 ml of Instagel (Packard Instrument Company) was added to aliquots of the superfusates and the tissue extracts and the radioactivity was counted. The values were corrected for efficiency and aliquot factors. The radioactivity released per impulse by nerve activity was calculated as a fraction of the total tissue content of radioactivity. The contractile response was quantified in terms of neurogenic increase in mean force (for details see Häggendal & others, 1970). In each experiment the values for fractional release of ^{3}H-NA and mechanical response during the second and the third period of stimulation were expressed in per cent of the corresponding responses for the initial stimulation period of the same experiment. The fractional release per impulse of the first stimulation period in the eight experiments amounted to $6·1 \times 10^{-5} \pm 0·5 \times 10^{-5}$ (mean $\pm$ s.e.).

Fig. 1 presents the relative values for fractional release and net mechanical response of the second and third stimulation period at 4 Hz. In normal Krebs solution the ^{3}H-NA release decreased whereas the mechanical responses were well maintained. In the preparations treated with PBZ 10^{-10}M the mean mechanical response was reduced to 58% (range 30–94%). The degree of reduction was more pronounced during the third stimulation period than in the second, which indicates an increasingly effective α-adrenoceptor blockade, whereas the ^{3}H-NA release was maintained at the same level (97% of control). At a PBZ concentration of 10^{-8}M the excitatory responses were blocked (three values 0, one value 7%) and the fractional release of ^{3}H-NA was increased to a value of 160% ($P < 0·05$, Student's *t*-test). When
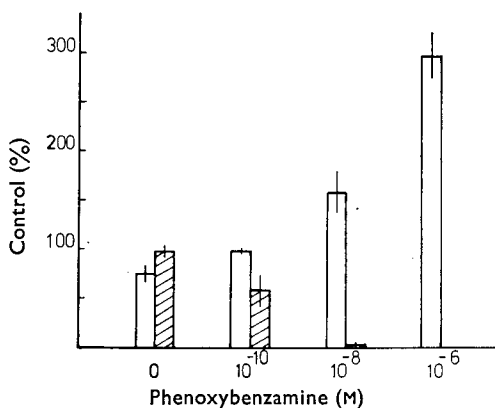

FIG. 1. Fractional ^{3}H-NA release per impulse and mean contractile responses induced by electrical field stimulation at 4 Hz of the rat isolated portal vein in normal Krebs solution and under influence of phenoxybenzamine (PBZ) 10^{-10}, 10^{-8} and 10^{-6}M. Each pair of bars represent values from four periods of stimulation in two experiments, expressed in % of initial control value. Note that the excitatory responses are practically abolished by PBZ 10^{-8}M but that the ^{3}H-NA output is further increased by PBZ 10^{-6}M. Mean $\pm$ s.e. is shown. Open columns; fractional release. Hatched columns; mechanical response.

the preparations were exposed to PBZ 10^{-6}M the transmitter release was further enhanced and reached a level of about 300% ($P <0.005$, Student's *t*-test). No excitatory responses were recorded but slight inhibitory effects could be seen suggesting β-adrenoceptor activation.

The experiments show that an effective α-adrenoceptor blockade, as judged by the abolishment of the excitatory responses, can be obtained by low concentrations of PBZ in the rat portal vein. The drug increases the amount of ^{3}H-NA output per impulse during nerve activity in a concentration-dependent manner, which seems especially pronounced after the PBZ concentration is increased above the level which is apparently required to achieve a complete α-adrenoceptor blockade.

Many α-adrenoceptor blocking drugs block the neuronal uptake mechanism, at least at high concentrations and this is often suggested to explain the increased overflow of noradrenaline after PBZ (see e.g., Gillespie & Kirpekar, 1966). However, efficient membrane pump blockers with insignificant α-adrenoceptor blocking effects, e.g., desipramine and LU 3-010 [1-phenyl-1-(3-methyl-aminopropyl)-3,3-dimethyl-phthalane] will in most preparations increase the transmitter overflow only moderately, if at all (Farnebo & Hamberger, 1970; Häggendal, 1970; Häggendal & others-unpublished findings). Furthermore, after the membrane pump blockers are given in apparently optimal doses, addition of α-adrenoceptor blocking agents will increase overflow materially (Geffen, 1965; Häggendal, 1970). Thus, inhibition of the membrane pump mechanism does not appear to be the major factor responsible for the increased release of noradrenaline following PBZ or other α-adrenoceptor blockers, even if a contribution to a minor extent cannot be excluded.

Extraneuronal uptake can occur at high concentrations of exogenous noradrenaline and it can be blocked by e.g., PBZ (Gillespie & Hamilton, 1966, 1967; Avakian & Gillespie, 1968). Extraneuronal uptake of noradrenaline released at nerve activity may also occur (cf. Iversen, 1971) and PBZ is also thought to block this extraneuronal uptake with the result that the metabolism of released noradrenaline is prevented (e.g., Langer, 1970). In the present study, however, the total radioactivity was followed, and therefore an effect of PBZ on the metabolism of ^{3}H-NA cannot explain the marked increase of the output of radioactivity. A blockade of extraneuronal uptake of noradrenaline by PBZ, thereby preventing a hypothetical tissue binding of the amine, is another explanation for the increased output of radioactivity. However, it seems doubtful that extraneuronal uptake and binding of noradrenaline released by nerve stimulation would be so large that a blockade of it could explain the three-fold increase in output of radioactivity found after PBZ (cf. Iversen, 1971).

Also a reduced or abolished fixation of noradrenaline to the α-adrenoceptors is unlikely to explain the results (see Boullin & others, 1967), since in the present experiments the output of noradrenaline was markedly increased at PBZ concentrations exceeding those necessary for complete α-adrenoceptor blockade.

It seems necessary to consider the possibility that the amount of transmitter released to the synaptic gap per nerve impulse may be increased after PBZ or other α-adrenoceptor blockers, either because of direct effects on the neurons or on local feed-back mechanisms, or both (cf. Häggendal, 1970). This possibility, an increased release of noradrenaline per nerve stimulus after PBZ, is also discussed by Farnebo & Hamberger (1970) and Langer (1970). In agreement with this view it recently has been found that PBZ increases not only the release of noradrenaline but also the release of the granular enzyme, dopamine-β-hydroxylase, at nerve stimulation (Put, De Potter & others, 1971). The present work shows that at a concentration of PBZ giving almost 100% blockade of α-adrenoceptors, the transmitter output was moderately increased, whereas a further increase of the PBZ concentration resulted in markedly increased output of radioactivity. Thus it appears that there is no

clearcut correlation between the degree of α-adrenoceptor blockade, as judged by the effector cell response, and the amount of transmitter released after PBZ. Other factors also appear to be contributing to the increase of transmitter amounts released at high PBZ concentration. This is also discussed by Starke, Montel & Wagner (1971), who have shown that the α-adrenoceptor blocking agent phentolamine potentiated the overflow of noradrenaline during nerve stimulation in rabbit isolated hearts. The authors concluded that if there is an enhancement of transmitter liberation per stimulus, the mechanism should not be dependent on α-adrenoceptor blockade.

PBZ is often used in high concentrations in quantitative studies on noradrenaline release, e.g., Stjärne & others (1969) and Langer & Vogt (1971) used PBZ of 3×10^{-5}M. The possibility of an increased transmitter output per nerve impulse cannot be excluded when PBZ in high concentrations is so used.

Departments of Pharmacology and Physiology,   JAN HÄGGENDAL
University of Göteborg,   BÖRJE JOHANSSON
Göteborg, Sweden.   JAN JONASON
          BENGT LJUNG

November 19, 1971

REFERENCES

AVAKIAN, O. M. & GILLESPIE, J. S. (1968). *Br. J. Pharmac. Chemother.*, **32**, 168–184.
BOULLIN, D. J., COSTA, E. & BRODIE, B. (1967). *J. Pharmac. exp. Ther.*, **157**, 125–134.
BROWN, G. L. & GILLESPIE, J. S. (1957). *J. Physiol., Lond.*, **138**, 81–102.
FARNEBO, L.-O. & HAMBERGER, B. (1970). *J. Pharm. Pharmac.*, **22**, 855–857.
GEFFEN, L. B. (1965). *J. Physiol., Lond.*, **181**, 67P–70P.
GILLESPIE, J. S. & HAMILTON, D. N. H. (1966). *Nature, Lond.*, **212**, 524–525.
GILLESPIE, J. S. & HAMILTON, D. N. H. (1967). *J. Physiol., Lond.*, **192**, 30P.
GILLESPIE, J. S. & KIRPEKAR, S. M. (1966). *Ibid.*, **187**, 51–68.
HÄGGENDAL, J. (1970). In: *New Aspects of Storage and Release Mechanisms of Catecholamines*, pp. 100–109. Bayer-Symposium II. Springer-Verlag.
HÄGGENDAL, J., JOHANSSON, B., JONASON, J. & LJUNG, B. (1970). *Acta physiol. scand., Suppl.* 349, 17–32.
IVERSEN, L. (1971). *Br. J. Pharmac.*, **41**, 571–591.
LANGER, S. Z. (1970). *J. Physiol. Lond.*, **208**, 515–546.
LANGER, S. Z. & VOGT, M. (1971). *J. Physiol., Lond.*, **214**, 159–171.
PUT, A., DE POTTER, W. P., CHUBB, I. W. & SCHAEPDRYVER, A. F. (1971). Presented at XXV Int. Congr. of Phys. Sci., Warsaw, Aug. 1971. In press.
STARKE, K., MONTEL, H. & WAGNER, J. (1971). *Arch. Pharmak.*, **270**, 210–214.
STÄRNE, L., HEDQVIST, P. & BYGDEMAN, S. (1969). *Life Sci.*, **8**, 189–196.
THOENEN, H., HÜRLIMANN, A. & HAEFELY, W. (1964). *Helv. Physiol. Pharmac. Acta*, **22**, 148–161.