Short communications

Enhancement of noradrenaline depletion in the cat spleen by phenoxybenzamine and phentolamine

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The noradrenaline content of the microsomal fraction of cat spleen was reduced by nerve stimulation. The reduction was greater in the presence of phenoxybenzamine and phentolamine, but was not affected by the presence of cocaine. The results suggest that phenoxybenzamine and phentolamine are able to increase the impulse-evoked release of noradrenaline.

Introduction.—In the blood-perfused cat spleen, phenoxybenzamine causes an increase in overflow of noradrenaline during nerve stimulation which is greater than the overflow which results from blocking uptakes I and II (Cripps & Dearnaley, 1971). It has been suggested that drugs which block α-adrenoceptors such as phenoxybenzamine are able to facilitate the impulse-evoked release of noradrenaline by affecting a local mechanism which regulates transmitter release (Hedqvist, 1969; Haggendal, 1970; Starke, Montel & Wagner, 1971; Kirpekar & Puig, 1971; Enero, Langer, Rothlin & Stefano, 1972). More recently, it has been shown that the overflow of dopamine-β-hydroxylase, a vesicular protein which is released along with noradrenaline from nerve terminals (de Potter, de Schaepdryver, Moerman & Smith, 1969; Geffen, Livett & Rush, 1969), is also proportionally increased by phenoxybenzamine and by phentolamine in the saline-perfused spleen and vas deferens during nerve stimulation (de Potter, Chubb, Put & de Schaepdryver, 1971; Johnson, Thoa, Weinshilboum, Axelrod & Kopin, 1971).

The vesicular noradrenaline of the cat spleen may be isolated in a microsomal fraction by subcellular fractionation procedures (Bisby & Fillenz, 1971). The depletion of microsomal noradrenaline in the stimulated spleen suggests that the vesicular noradrenaline makes the greatest contribution to transmitter release (Bisby, Cripps & Dearnaley, 1971; Fillenz & Howe, 1971). In the present experiments, the reduction in noradrenaline concentration of the microsomal fraction of the cat spleen has been measured after nerve stimulation. The reduction obtained in cats which were pretreated with cocaine, phenoxybenzamine, or phentolamine are compared with the reduction obtained in untreated cats, in order to see whether the presence of these drugs is able to enhance the reduction of microsomal noradrenaline content following nerve stimulation.

Methods.—Cats of either sex were prepared under halothane anaesthesia for *in situ* stimulation of the splenic nerve via electrodes placed on the main nerve trunk as described by Dearnaley & Geffen (1966). Phenoxybenzamine (10 mg/kg), phentolamine (10 mg/kg), and cocaine (2 mg/kg) were given intravenously to some of the cats at 20 min, 15 min, and 5 min before stimulation respectively. Immediately after removal of a control portion of the spleen, the splenic nerve received a train of 1,000 stimuli at 10 Hz (20 V pulses of 0·5 ms duration). The remainder of the spleen was rapidly removed and both portions were transferred to ice-cold 0·3 M sucrose/ethylene diamine tetra-acetic acid solution, homogenized, then subjected to differential centrifugation as described previously (Bisby & Fillenz, 1971). The microsomal pellets thus prepared were resuspended in perchloric acid and the content of noradrenaline and protein was assayed chemically (Bisby & Fillenz, 1971).

In a few experiments, the venous effluent from the spleen was collected during stimulation, treated in the same way as tissue samples, and the microsomal protein content was determined.

Results.—With only one exception, the noradrenaline/protein concentration in the microsomal fraction of the stimulated portion of spleen was less than that of the unstimulated portion. Nerve stimulation in the presence of cocaine (Fig. 1), reduced the noradrenaline/protein concentration in the microsomal fraction to 78·0 ± 5·4% (mean ± S.E.M. of 4 experiments). This is

not significantly different ($P>0.25$) from the reduction to $83.6\pm7.8\%$ ($n=5$) which was obtained without drug treatment.

With phenoxybenzamine, however, the noradrenaline/protein concentration in the microsomal fraction was reduced to $44.0\pm1.4\%$ ($n=4$) by stimulation and, with phentolamine, to $63.6\pm4.0\%$ ($n=5$). Each of these reductions is significantly greater than the reduction obtained with either no drug treatment or cocaine pretreatment ($P<0.01$ with phenoxybenzamine; $P<0.05$ with phentolamine). The depletion of microsomal noradrenaline by nerve stimulation in the presence of phenoxybenzamine was 2·5 times that obtained with cocaine, and 3·4 times that obtained without drug treatment. The depletion in the presence of phentolamine was 2·2 times that obtained without drug treatment.

The microsomal protein which was found in the venous effluent following stimulation represented a maximum possible loss of 5%-6% of the total microsomal protein content of the stimulated portion of the spleen.

Discussion.—Loss of vesicular noradrenaline due to impulse-evoked release from nerve terminals is thought to be compensated for by re-uptake of noradrenaline and synthesis of noradrenaline. However, the depletion of microsomal noradrenaline in the cat spleen after nerve stimulation is not significantly greater in the presence of cocaine, which is a potent inhibitor of neuronal uptake (Iversen, 1967). This suggests that, during continuous stimulation at 10 Hz, neuronal uptake in the spleen may become saturated.

Blakeley, Brown, Dearnaley & Harrison (1968) found that the rate of noradrenaline synthesis in the blood-perfused spleen during intermittent nerve stimulation ranged from 6% to 29% of the total spleen noradrenaline content per hour. This suggests that synthesis of noradrenaline during the 100 s stimulation period used in the present experiments would probably be negligible. If the contribution of synthesis is neglected, then the depletion of microsomal noradrenaline in the present experiments may be regarded as an index of transmitter release, provided that the re-uptake of vesicular noradrenaline is effectively blocked.

By comparing the depletion obtained in the presence of cocaine with the depletion in the presence of phenoxybenzamine, which also blocks neuronal uptake (Thoenen, Hurlimann & Haefely, 1964), it appears that phenoxybenzamine greatly

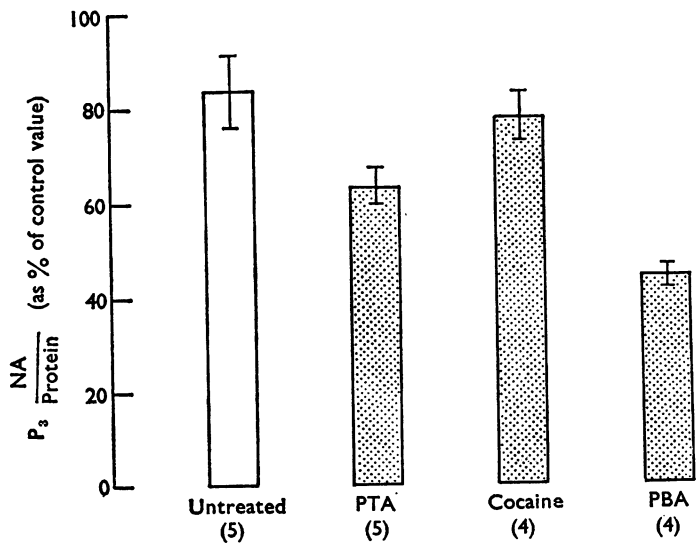

FIG. 1. Noradrenaline (NA) concentration in microsomal fraction of cat spleen after nerve stimulation (1,000 stim. at 10 Hz). Effects of pretreatment with phentolamine (PTA), cocaine, and phenoxybenzamine (PBA). The numbers of experiments in each case are given in parentheses.

increases the release of vesicular noradrenaline by nerve stimulation. Since phentolamine does not block neuronal uptake of noradrenaline (Hertting, Axelrod & Whitby, 1961), the depletion obtained in the presence of phentolamine may be compared with the release of noradrenaline by nerve stimulation in the absence of drug treatment. It appears that phentolamine is also able to increase the impulse-evoked release of vesicular noradrenaline, but to a lesser extent than phenoxybenzamine.

Dearnaley & Geffen (1966) reported that contraction of the stimulated spleen results in a loss of blood. This might have caused a reduction in the microsomal noradrenaline/protein concentration to be underestimated. However, it appears that the protein lost in the venous effluent during stimulation was mainly soluble. Since the maximum possible loss of microsomal protein during stimulation was only 5%-6%, the reduction in noradrenaline concentration of the untreated or cocaine-treated spleens may have been underestimated by no more than 1·3%. This error did not arise in the phenoxybenzamine- or phentolamine-treated spleens where contraction of the spleen during stimulation was prevented by α-adrenoceptor blockade.

In summary, both phenoxybenzamine and phentolamine enhanced the depletion of microsomal noradrenaline in the cat spleen in response to nerve stimulation, whereas cocaine did not. These observations were not affected by errors due to contraction of the stimulated portion of the spleen. They suggest that phenoxybenzamine and, to a lesser extent, phentolamine are able to increase the impulse-evoked release of vesicular noradrenaline in the cat spleen.

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