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Effect of β-haloalkylamines and ephedrine on noradrenaline release from the intact spleen of the cat

S. M. KIRPEKAR AND A. R. WAKADE

Department of Pharmacology, State University of New York, Downstate Medical Center, Brooklyn, New York

Summary

1. The effects of β-haloalkylamines, phenoxybenzamine (PBZ), N-α-naphthylmethyl-N-ethyl-β-bromoethylamine (SY28), N-cyclohexyl-methyl-N-ethyl-β-chloroethylamine (GD131) and ephedrine on noradrenaline (NA) output following nerve stimulation were studied in the intact spleen of the cat. Postganglionic sympathetic nerves were stimulated at frequencies of 10 and 30 Hz for a total of 210 stimuli.

2. PBZ and SY28 (10 mg/kg) increased the transmitter output at 10 Hz by nearly 5–10 times.

3. Administration of GD131 (10 mg/kg) had no effect on output at either frequency. Phentolamine (3 mg/kg) given after GD131 increased NA output at 10 Hz.

4. Ephedrine (0·5 mg/kg) caused no changes in catecholamine output, while higher doses of ephedrine (10–20 mg/kg) increased the amount released at 10 Hz.

5. Perfusion of the spleen with Krebs-bicarbonate solution containing either SY28, GD131 or ephedrine (10 μg/ml) increased the recovery of infused NA by 80–90%.

6. It is concluded that presynaptic as well as postsynaptic events determine the overflow of NA following stimulation of sympathetic nerves of the spleen.

Introduction

Brown & Gillespie (1957) found that after treatment with phenoxybenzamine (PBZ), stimulation of the splenic nerves of cats at low frequencies resulted in a greater "overflow" of the adrenergic transmitter in the venous blood. As PBZ is a potent irreversible blocking agent of α-adrenoceptors, they suggested that these receptors are responsible for the inactivation of neurally released noradrenaline (NA). However, increase in NA output following administration of PBZ could also have been due to the ability of this agent to prevent the re-uptake of neurally released NA by adrenergic nerve terminals, since it is known to block effectively the uptake of exogenous NA (Hertting, Axelrod & Whitby, 1961; Furchgott & Kirpekar, 1963; Gillespie & Kirpekar, 1965). On the other hand, it was shown that cocaine, which also can block uptake of exogenous NA, increased NA output

from the spleen only slightly at low frequencies of stimulation; whereas phentolamine, which has no effect on uptake (Hertting *et al.*, 1961), markedly enhanced NA output (Kirpekar & Cervoni, 1963; Blakeley, Brown & Ferry, 1963).

The present experiments were undertaken to investigate further the role played by the α-adrenoceptors of the spleen in the inactivation of neurally released NA. We have used three related β-haloalkylamines: PBZ, N-α-naphthylmethyl-N-ethyl-β-bromoethylamine (SY28), and N-cyclohexyl-methyl-N-ethyl-β-chloroethylamine (GD131), because these agents have been previously shown to vary in their potencies as blocking agents of α-adrenoceptors and as blocking agents of the neuronal uptake mechanism for NA (Furchgott & Kirpekar, 1963). As blocking agents for α-adrenoceptors of rabbit aortic strips, the relative order of potency is PBZ>SY28$\gg$GD131. As blocking agents of neuronal uptake mechanism, as judged by sensitization of guinea-pig atria to NA, the relative order of potency is PBZ>GD131>SY28. If uptake of NA were the only factor determining the overflow in the venous blood, then GD131 should be more effective than SY28 in releasing NA following sympathetic nerve stimulation. In addition to investigating the effects of β-haloalkylamines on transmitter release, we have investigated the effects of ephedrine since Gaddum & Kwiatkowski (1938) and Ambache (1951) showed that this agent in higher concentrations antagonized the response to either nerve stimulation or adrenaline.

Methods

Cats were anaesthetized with ether followed by chloralose (40–60 mg/kg). The abdomen was opened by a midline incision and the stomach, intestines and colon were removed. Both adrenals were removed. Arrangements for splenic nerve stimulation and blood sample collection were similar to those previously described by Brown & Gillespie (1957). Noradrenaline content of the plasma was measured biologically on the blood pressure of a pithed rat. Splenic nerves were ligated centrally and stimulated at 10 Hz or 30 Hz for a total of 210 stimuli. Stimulation intensity was 25 V and duration was 1 ms.

After obtaining the control samples at stimulation frequencies at 10 and 30 Hz, β-haloalkylamines were given intravenously and the stimulation samples were collected after about 30 min. After ephedrine and phentolamine 10–20 min were allowed before nerves were stimulated. The interval between stimulations was about 10 min. In a few experiments the spleen was perfused with Krebs-bicarbonate solution at a rate of 7 ml/min while noradrenaline was infused into the spleen by placing a cannula into the hepatic artery so that the tip was just short of the junction with the splenic artery. The procedure used to determine the inactivation of infused NA was similar to that described by Gillespie & Kirpekar (1965).

Results

Effect of PBZ on NA output from spleen

Postganglionic sympathetic nerves of the cat's spleen were stimulated at frequencies of 10 and 30 Hz for a total of 210 stimuli. These frequencies were selected in view of the previous observations (Brown & Gillespie, 1957; Trendelenburg, 1959; Kirpekar & Cervoni, 1963) that the transmitter output following stimulation

at the higher frequency was maximal, whereas it was barely detectable when splenic nerves were stimulated at the lower frequency of 10 Hz. Figure 1 shows the effect of pretreatment with PBZ (10 mg/kg) on NA output at 10 and 30 Hz. It is readily apparent that the transmitter output at 10 Hz was markedly enhanced following treatment with PBZ. This observation confirms the original findings of Brown & Gillespie (1957).

Effect of SY28 on NA output from spleen

SY28 is a potent blocking agent of α-adrenoceptors, about one-tenth as potent as PBZ on rabbit aortic strips (R. F. Furchgott, personal communication). A typical experiment showing the effect of SY28 on NA release is illustrated in Fig. 2. SY28 was given intravenously in a single injection at a concentration of 10 mg/kg. Following administration of SY28, the transmitter output at 10 Hz was markedly increased to a level comparable with the control sample at 30 Hz. Subsequent stimulation at 10 Hz resulted once again in a very marked increase in NA output. In five experiments, control NA outputs at 10 and 30 Hz were $0{\cdot}18 \pm 0{\cdot}03$ and $0{\cdot}80 \pm 0{\cdot}13$ ng/stimulus respectively. After treatment with SY28 the respective outputs were $1{\cdot}71 \pm 0{\cdot}4$ and $1{\cdot}09 \pm 0{\cdot}23$ ng/stimulus. Thus SY28 produced an increase in transmitter output at the lower frequency of stimulation in a manner similar to PBZ.

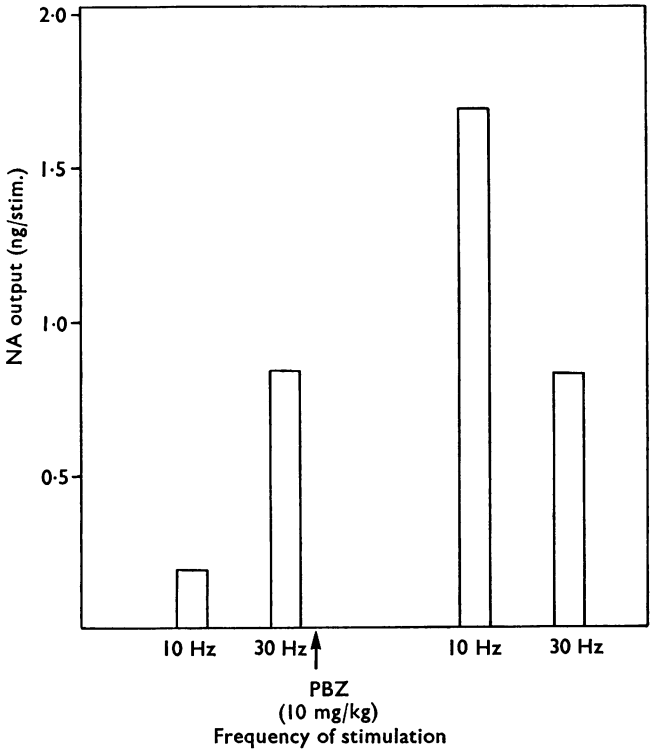

FIG. 1. Effect of PBZ on NA output from spleen. NA values are expressed as ng/stimulus. PBZ (10 mg/kg) was given intravenously and the first 10 Hz sample was collected 30 min later. Time interval between successive samples was approximately 10 min.

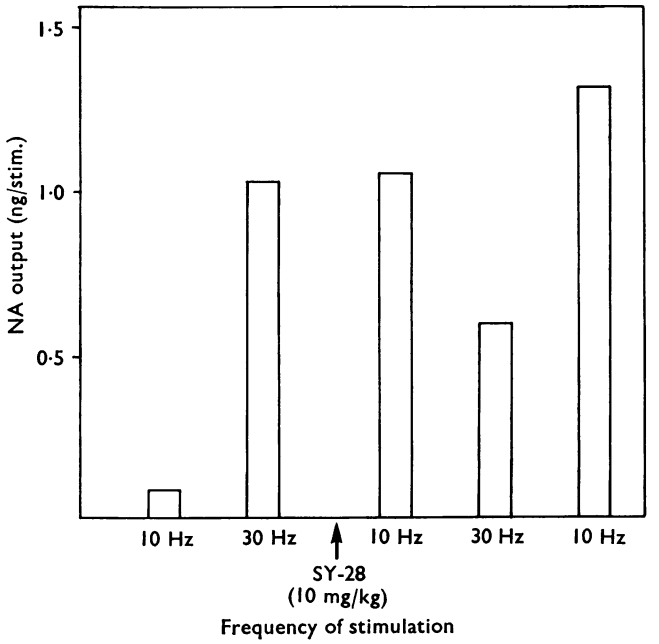

FIG. 2. Effect of SY28 on NA output from spleen. SY28 (10 mg/kg) was given intravenously in a single injection and the first 10 Hz sample was collected 30 min later. Time interval between successive samples was approximately 10 min.

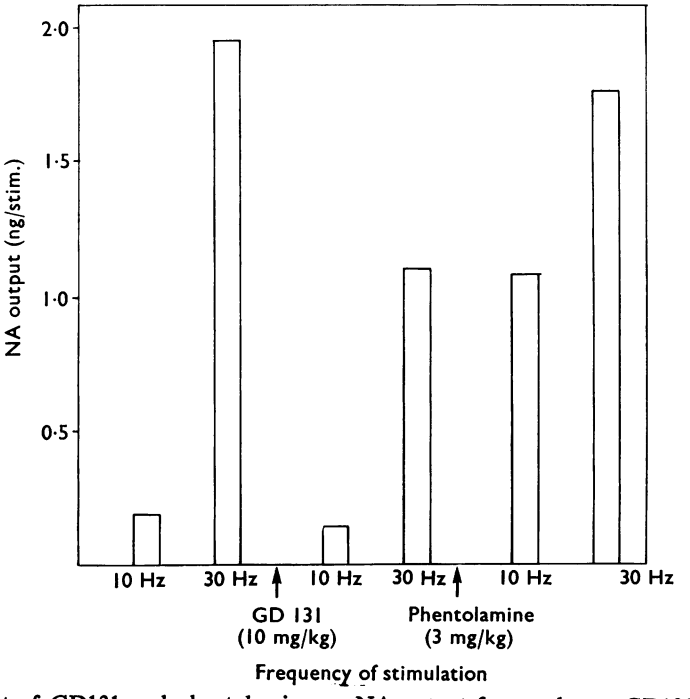

FIG. 3. Effect of GD131 and phentolamine on NA output from spleen. GD131 (10 mg/kg) was given intravenously and the first 10 Hz sample was collected 30 min later. Phentolamine (3 mg/kg) was given intravenously and the sample at 10 Hz was collected 10 min later. Time interval between successive samples was approximately 10 min.

Effect of GD131 on NA output from spleen

GD131 is a very weak blocking agent for α-adrenoceptors, only about one ten-thousandth as potent as PBZ on aortic strips (R. F. Furchgott, personal communication). An experiment is illustrated in Fig. 3 which shows the effect of GD131 (10 mg/kg) on transmitter output. GD131 had no effect on NA output at the frequency of 10 Hz. At 30 Hz the NA output was reduced after treatment with GD131 as compared with the control output at the same frequency of stimulation. In other experiments of this type there was only a small increase in NA release after this agent when splenic nerves were stimulated at 10 Hz. A more important observation is recorded after treatment with phentolamine (3 mg/kg). After a 10 min interval the response to stimulation at 10 Hz was much greater than the control level at 10 Hz, while the response at 30 Hz was similar to the control at 30 Hz. Following administration of phentolamine the NA output at 10 and 30 Hz was affected in a manner similar to PBZ.

In seven experiments, control NA outputs at 10 and 30 Hz were $0{\cdot}17 \pm 0{\cdot}06$ and $1{\cdot}11 \pm 0.33$ ng/stimulus. After treatment with GD131, the respective outputs were $0{\cdot}23 \pm 0{\cdot}044$ and $0{\cdot}79 \pm 0{\cdot}13$ ng/stimulus.

Effect of ephedrine on NA output from spleen

Gaddum & Kwiatkowski (1938) showed that ephedrine increased NA output from rabbit ear following stimulation of postganglionic sympathetic fibres. This increase in transmitter output was attributed partially to the monoamine oxidase-inhibiting ability of ephedrine. We have studied the effect of ephedrine on NA output from spleen, using both low and high concentrations. Figure 4 shows the effect of 0·5 mg/kg of ephedrine, and it is quite evident that the pattern of NA release at frequencies of 10 and 30 Hz did not change after ephedrine. Once again, administering

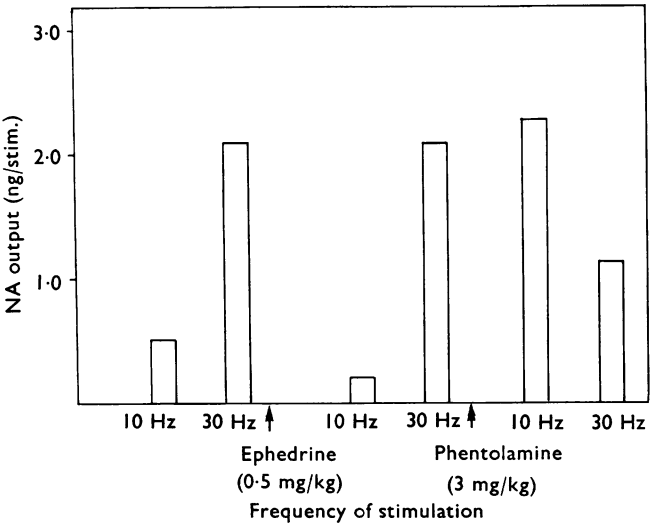

FIG. 4. Effect of ephedrine and phentolamine on NA output from spleen. Ephedrine (0·5 mg/kg) was given intravenously in a single injection and the first 10 Hz sample was obtained about 10 min later. Phentolamine (3 mg/kg) was then given intravenously and the 10 Hz sample obtained 10 min later. Time interval between successive samples was approximately 10 min.

phentolamine resulted in a very marked increase in NA output at 10 Hz. In three experiments, control NA outputs at 10 and 30 Hz were $0{\cdot}36 \pm 0{\cdot}14$ and $1{\cdot}16 \pm 0{\cdot}49$ ng/stimulus respectively. After ephedrine (0·5 mg/kg) the respective outputs were $0{\cdot}21 \pm 0{\cdot}003$ and $1{\cdot}57 \pm 0{\cdot}51$ ng/stimulus.

Figure 5 shows the effect of a high dose of ephedrine on NA release. After collecting samples at 10 and 30 Hz, ephedrine (20 mg/kg) was given in a single injection. This resulted in a very marked contraction of the spleen, which remained contracted for the duration of the experiment. The splenic nerves were stimulated 20 min later at 10 Hz. After this large dose of ephedrine, the background pressor

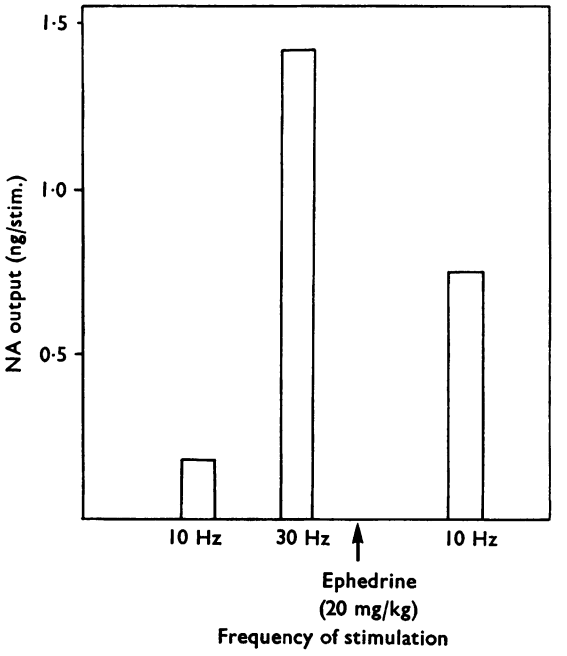

FIG. 5. Effect of a high dose of ephedrine on NA output from spleen. Ephedrine (20 mg/kg) was given intravenously in a single injection and the 10 Hz sample was obtained 20 min later. Time interval between successive samples was approximately 10 min.

TABLE 1. *Effect of SY28, GD131 and ephedrine on the recovery of NA in the splenic venous perfusate during arterial infusion of NA*

Cat. No.	Treatment*	NA infused in 60 s (ng)	NA recovered in 60 s (ng) 10 min	15 min	20 min	Mean recovery in 10, 15 and 20 min samples	Recovery %
22	SY28 (10 μg/ml)	510	547	547	547	547	107
24	,, ,,	510	490	507	507	501	98
23	GD131 (10 μg/ml)	510	533	533	547	538	105
25	,, ,,	510	486	480	486	484	95
28	,, ,,	510	434	432	473	443	87
29	,, ,,	510	442	436	436	438	86
30	,, ,,	510	460	473	488	474	93
36	Ephedrine (10 μg/ml)	510	400	440	400	413	81

* Perfusion was started approximately 30 min before and was continued during infusion of NA. In previous experiments using the same procedure, the NA recovery in normal spleens was 34% (Kirpekar & Wakade, 1968).

activity of the plasma was raised to 15 ng/ml. Output of NA at 10 Hz was also considerably increased. In six experiments the mean control output at 10 Hz was 0·16 ng/stimulus. After treatment with ephedrine (10 or 20 mg/kg) this was raised to 0·616 ng/stimulus. It should be pointed out, however, that the ability of adrenergic blocking agents to increase the transmitter output at the lower frequency of stimulation was always much greater than ephedrine.

Effect of SY28, GD131 and ephedrine on the recovery of NA in the splenic venous perfusate during arterial infusion of NA

NA was injected into the spleen via a cannula in the hepatic artery at a constant infusion rate of 0·510 μg/min. Venous samples were collected at 10, 15 and 20 min periods of infusion. Results are shown in Table 1. Kirpekar & Wakade (1968) previously showed that 34% of infused NA was recovered in the venous perfusate. Perfusing the spleen with 10 μg/ml of either SY28, GD131 or ephedrine increased the NA recovery to 80–90%. At these concentrations, both these agents prevented the uptake of NA quite effectively, in a manner similar to PBZ (Gillespie & Kirpekar, 1965).

Discussion

GD131, as compared with PBZ and SY28, is a very weak blocking agent of α-adrenoceptors in rabbit aorta, being only about one ten-thousandth as potent as PBZ and about one-thousandth as potent as SY28. On the other hand, studies on the relative potencies of these agents in sensitizing isolated guinea-pig atria to NA leads to the conclusion that GD131 is about one-tenth as potent as PBZ in blocking neuronal uptake of NA, and slightly more potent than SY28 in this regard (R. F. Furchgott, personal communication). Because of these potency variations, GD131, unlike PBZ and SY28, might be expected to block the uptake mechanism effectively before blocking α-adrenoceptors if used in appropriate concentrations.

We have shown that PBZ and SY28 consistently increased the transmitter output from the spleen at the lower rate of stimulation so that the transmitter outputs at 10 and 30 Hz became comparable ; whereas GD131 did not increase the output of NA when administered in the same dose as the other two agents. All three agents, however, prevented the uptake of infused NA. Gillespie & Kirpekar (1965) previously showed that, of the NA infused intra-arterially in the presence of PBZ, nearly 80–85% was recovered in the splenic venous blood, thereby showing an effective blockade of the uptake of infused NA by PBZ. In the present series of experiments, when NA was infused in the presence of SY28 or GD131 the NA recovery was over 80–90%. We concluded from these observations that although all the three β-haloalkylamines were effective in preventing the uptake of infused NA in the spleen, their ability to increase the transmitter output, following splenic nerve stimulation at the lower frequency, did not appear to be related to this property. On the other hand, the increase in transmitter output could be related to the ability of these compounds to block the adrenoceptors in spleen.

The present experiments therefore support the view put forward by Brown & Gillespie (1957) and Gillespie & Kirpekar (1965) that the receptor has a vital role in the reabsorption of neurally released transmitter from spleen by acting as a " brake " on the diffusion away from the nerve endings. (For a similar view see also

Boullin, Costa & Brodie, 1967.) It would seem rather difficult to assume that all α-adrenoceptor blocking agents which consistently increase the transmitter output at lower frequencies would also be potent inhibitors of NA uptake in sympathetic nerve endings. As a matter of fact, Hertting *et al.* (1961) have shown that phentolamine, which is a potent α-adrenoceptor blocking agent, does not prevent NA uptake either in spleen or heart and yet increases NA overflow.

Experiments with ephedrine were carried out, because pharmacological evidence indicates that this agent at high concentrations can compete with NA for α-adrenoceptors (Gaddum & Kwiatkowski, 1938; Furchgott, 1955). It was clear that ephedrine in concentrations of 10 mg/kg or more enhanced NA outflow in response to nerve stimulation to a certain extent. Increase in transmitter output on nerve stimulation in the presence of ephedrine is probably due to partial occupation of α-adrenoceptors by this amine and/or its ability to prevent NA uptake. It should be pointed out, however, that agents such as cocaine and desmethylimipramine (Geffen, 1965), which are far more potent than ephedrine in preventing uptake of exogenous NA, do not consistently increase transmitter output at lower frequencies of stimulation.

Avakian & Gillespie (1968) have shown uptake of exogenous NA into smooth muscle cells of spleen and blood vessels after exposure to high NA concentrations, which was blocked by PBZ. They have suggested that "uptake$_2$" (Iversen, 1965) may be a physiological process involved in the inactivation of high concentrations of NA released at the nerve endings. Iversen & Langer (1969) have suggested that the effects of PBZ on overflow of NA following nerve stimulation cannot be explained only on the ability of PBZ to prevent uptake of NA by nerve terminals. They have concluded that prevention of metabolism of the released transmitter by uptake might be an important contributory factor.

The object of the present investigation is not to belittle the role of sympathetic nerves to inactivate neurally-released transmitter. Post-ganglionic sympathetic nerves are the principal sites for NA inactivation, but the facilitatory role of the receptor in this reabsorption process should also be considered. It is not clear whether the receptor plays a direct role in presenting NA back to the nerves or whether, by producing generalized contraction, it imposes a temporary restriction on the transmitter to diffuse away from its site of release and thus facilitate reabsorption.

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