Biochemical Pharmacology, Vol. 27, 1512–1513.

0006-2952/78/0515-1512/$02.00/0

Reversal of bretylium blockade of adrenergic nerve terminals by tetraethylammonium

(*Received* 14 *July* 1977; *accepted* 19 *September* 1977)

Several proposals have been made to explain the neuronal blocking action of bretylium. Cabrera *et al.* [1] showed that bretylium inhibits the acetylcholine (ACh)-induced antidromic discharges originating in the terminal parts of the C fibers. They suggested a possible action of bretylium on the excitability of the terminal portion of the nerve membrane. A similar suggestion was made by Haeusler *et al.* [2], who proposed that bretylium selectively accumulates intraneuronally and acts as a local anesthetic on the membrane of the adrenergic nerve terminals, thus inhibiting norepinephrine (NE) release. A different proposal, that bretylium interferes with stimulus–secretion coupling, was made by Davey *et al.* [3], who showed that bretylium, while blocking the ACh-induced release of NE from the cat spleen, did not block ACh-induced antidromic firing in splenic C fibers. The present investigation was undertaken to test the proposal that bretylium may interfere with some step in stimulus–secretion coupling and to determine whether tetraethylammonium (TEA), which enhances NE output from adrenergic nerves—presumably by increasing calcium entry (4–6)—could reverse the neuronal blockade produced by bretylium.

Cats (about 2 kg each) were anesthetized with ether, followed by chloralose (60 mg/kg, i.v.). The arrangements for perfusion of the spleen *in situ* were similar to those previously described [7]. The spleen was perfused with Krebs–bicarbonate solution at 35° by means of a pump (Sigmamotor, model A14E) at a constant rate of about 7 ml/min. The solution was bubbled with 95% O_2–5% CO_2, and the final pH was 7.4 to 7.5. The splenic nerves were stimulated with rectangular pulses (25 V, 1 msec) at a frequency of 10 Hz for 20 sec. Each venous perfusate sample was collected for 2 min, beginning with the start of stimulation. Perfusion pressure was recorded as a measure of peripheral resistance. The NE content of the venous samples was determined fluorometrically [8].

The spleen was initially perfused with drug-free Krebs solution for 30 min, followed by Krebs solution containing bretylium (5 μg/ml) for 15 min. After the inhibitory effect of bretylium on NE release was established, perfusion was continued with Krebs solution containing TEA (1 mM) for about 60 min and then with drug-free Krebs solution for an additional 30–60 min. During the different perfusion periods the nerves were stimulated at intervals of 15 or 30 min.

NE output from the perfused cat spleen remained fairly constant during repeated nerve stimulation [6]. It has been reported that the inhibitory effect of bretylium on the release of NE induced by nerve stimulation persists even after the removal of bretylium from the perfusion fluid [9]. We did two experiments to confirm that the inhibitory effect of bretylium on release persists for more than 1 hr after washout of the drug. The spleen was first perfused with bretylium to block the release of NE. After a 90-min perfusion with bretylium-free solution, the NE output at 10 Hz was only 23 per cent of the initial control output.

Figure 1 shows that TEA reverses the inhibitory effect of bretylium on NE output. Bretylium suppressed NE outflow induced by nerve stimulation by about 80 per cent. After a 30-min perfusion with TEA (1 mM) solution, the NE output was restored to the initial control output. As perfusion with TEA solution continued, the output increased so that with the second stimulation it was about 1.5 times greater than the control value. Perfusion pressure responses to nerve stimulation followed roughly the same pattern as NE release. Thus, after perfusion with bretylium these responses were greatly suppressed, whereas perfusion with TEA solution not only restored the pressure

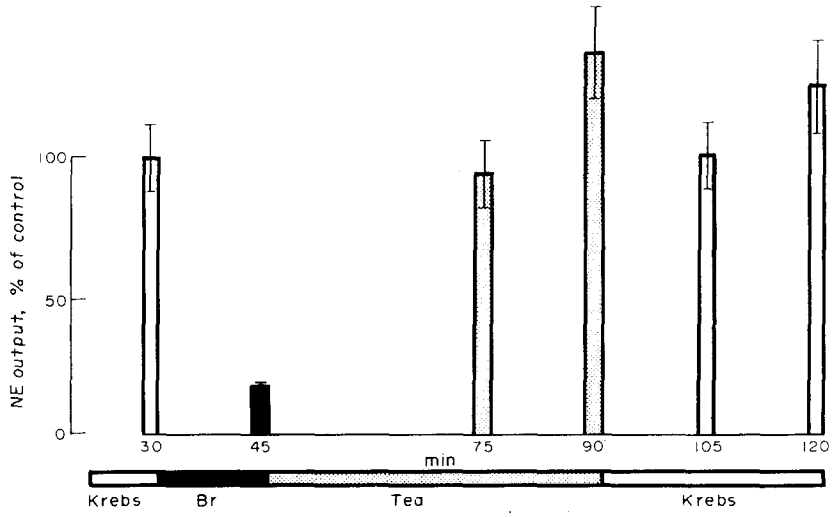

Fig. 1. Effect of TEA on the inhibitory effect of bretylium (Br) on NE output from the perfused spleen *in situ*. Splenic nerves were stimulated with rectangular pulses (25 V, 1 msec) at 10 HZ for 20 sec. NE output is expressed as percentage (mean ± S.E.) of the initial output (= 100 per cent) in six experiments. First column: □, initial output 158 ± 20 ng/2 min; Second column: ■, perfusion with bretylium (5 μg/ml); Third and fourth columns: □, perfusion with TEA (1 mM).

reponses fully but even potentiated them. On final reperfusion with drug-free Krebs solution, the mean NE output on the first and second stimulation was 102 and 126 per cent, respectively, of the initial control value. In two experiments, the stimulations were repeated two more times, and the output was 107 and 110 per cent of the control value. The corresponding perfusion pressure responses were also restored to control levels.

Experiments reported in this communication show that TEA reversed the inhibitory effect of bretylium on NE output from the spleen by nerve stimulation. It has been shown previously that guanethidine blockade of adrenergic nerve terminals is also reversed by TEA, but only temporarily, since on reperfusion with normal Krebs solution the blockade gradually reappears [10]. However, the reversal of bretylium blockade by TEA appears to be permanent, since adrenergic neurotransmission was restored not only during perfusion of the spleen with TEA, but also during a subsequent perfusion with a drug-free solution.

Since bretylium blockade is removed permanently by TEA, it is possible that TEA somehow displaces bretylium from the adrenergic nerve terminals and restores transmission. One possibility is that, because both TEA and bretylium have a quaternary ammonium group, the former may displace the latter from its binding sites. Alternatively, TEA is known to greatly increase NE release by nerve stimulation, presumably by enhancing calcium uptake into the neurons [4–6], and it may be that excess calcium accumulating in the nerves is partly responsible for reversing the inhibitory action of bretylium on NE release. Previously, Burn and Welsh [11] showed that high calcium counteracted the blocking action of bretylium on the relaxation of the isolated rabbit ileum produced by stimulation of its periarterial nerves.

Department of Pharmacology, State University of New York, Downstate Medical Center, Brooklyn, NY 11203, U.S.A.

MADHURI KIRPEKAR
SADASHIV M. KIRPEKAR
JOHN C. PRAT

REFERENCES

1. R. Cabrera, R. W. Torrance and H. Viveros, *Br. J. Pharmac. Chemother.* **27**, 51 (1966).
2. G. Haeusler, W. Haefely and A. Huerlimann, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **265**, 260 (1969).
3. M. J. Davey, M. L. Hayden and P. C. Scholfield, *Br. J. Pharmac. Chemother.* **39**, 377 (1968).
4. H. Thoenen, W. Haefely and H. Staehelin, *J. Pharmac. exp. Ther.* **157**, 532 (1967).
5. S. M. Kirpekar, J. C. Prat, M. Puig and A. R. Wakade, *J. Physiol., Lond.* **221**, 601 (1972).
6. S. M. Kirpekar, A. R. Wakade and J. C. Prat, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **294**, 23 (1976).
7. S. M. Kirpekar and Y. Misu, *J. Physiol., Lond.* **188**, 219 (1967).
8. A. H. Anton and D. F. Sayre, *J. Pharmac. exp. Ther.* **138**, 360 (1962).
9. H. Thoenen, A. Huerlimann and W. Haefely, *J. Pharmac. exp. Ther.* **151**, 189 (1966).
10. M. Kirpekar, S. M. Kirpekar and J. C. Prat, *Fedn Proc.* **36**, 327 (1977).
11. J. H. Burn and F. Welsh, *Br. J. Pharmac. Chemother.* **31**, 74 (1967).