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CAN BARIUM SUPPORT THE RELEASE OF ACETYLCHOLINE BY NERVE IMPULSES?

E. M. SILINSKY

Department of Pharmacology, Northwestern University Medical School, 303 E. Chicago Avenue, Chicago, Illinois 60611, U.S.A.

Conventional electrophysiological techniques were used to study the effects of Ba on the release of acetylcholine (ACh) from frog motor nerve terminals. Equimolar substitution of Ba for Ca eliminated end-plate potentials (e.p.ps) without a corresponding decline in the amplitude of the nerve terminal action potential. Miniature end-plate potentials (m.e.p.ps) were readily detectable in Ba solutions despite a depolarized muscle membrane. Studies on the e.p.p in curarized preparations bathed with different concentrations of Ca and Ba suggest that Ba may compete with Ca in the process by which depolarization of the nerve terminal leads to the release of ACh. Repetitive nerve stimulation at 1 Hz in Ba solutions caused 5–20 fold increases in m.e.p.p frequencies (7 experiments). Stimulation of Ba-bathed preparations at 10 Hz elevated m.e.p.p frequencies to very high levels that could not be measured accurately (» 100/s). It is suggested that the asynchronous discharge of m.e.p.ps produced by repetitive nerve stimulation is the electrophysiological correlate of the evoked ACh outflow in Ba solutions detected previously by bioassay of the perfusion fluid.

Introduction In studies on cat superior cervical ganglion, Ba has been shown to be an effective substitute for Ca in supporting the release of acetylcholine (ACh) by nerve impulses when transmitter output was measured by bioassay of the perfusion fluid (see e.g. Douglas, Lywood & Straub, 1961). These results appear somewhat contradictory to the few reports in the electrophysiological literature which suggest that Ba is an extremely poor substitute for Ca in supporting the evoked release of ACh. For example, the results of Miledi (1966) and Blioch, Glagoleva, Liberman & Nenashev (1968) with frog motor nerve terminals suggest that end-plate potentials (e.p.ps) are only occasionally detectable in Ba solutions, and when produced involve the release of a very small fraction of the quantal output normally observed in Ca solutions. Several reviewers (Hubbard, 1973; Ginsborg & Jenkinson, 1976) have in fact suggested that these small e.p.ps reflect the transient displacement of membrane Ca by Ba (Laskowski & Thies, 1972), Ba in itself being ineffective in the process of evoked ACh release. This study was undertaken in an attempt to resolve this discrepancy and to provide an electrophysiological correlate of the release of ACh into the effluent from stimulated nerve terminals bathed in Ba solutions.

Methods The isolated nerve-cutaneous pectoris preparation of the frog was bathed in normal Ringer solution of the following composition (mM): NaCl, 115, KCl 2, $NaHCO_3$ 2 and $CaCl_2$ 1.8. Ba Ringer solution was identical except that 1.8 mM $BaCl_2$ was substituted for $CaCl_2$. Ba-EGTA Ringer solution contained 3 mM $BaCl_2$, 1 mM ethyleneglycol-*bis*(β-aminoethylether)-*N,N'*-tetra-acetic acid (EGTA) and no added Ca, the remaining salts being identical to normal Ringer. Neostigmine methyl sulphate (1–10 μg/ml) and tubocurarine chloride (2.5 μg/ml) were used in some experiments. Solutions were changed by a roller pump (for protocol see Silinsky, 1974). The responses to supramaximal nerve stimulation were recorded intracellularly by conventional methods (Fatt & Katz, 1951). For focal recording of nerve terminal action potentials and e.p.ps, end-plate regions were localized by recording miniature end-plate potentials (m.e.p.ps) extracellularly (Hubbard & Schmidt, 1963). After amplification, signals were fed in parallel into a computer for average transients (Fabritek) and a pen recorder (Brush-Gould). For determining e.p.p. amplitudes, responses to 128 nerve stimuli were averaged and displayed on a storage oscilloscope. M.e.p.p. frequencies were determined from pen records.

Results Figure 1a (upper trace) demonstrates that equimolar substitution of Ba for Ca eliminates the e.p.p. without impairing conduction of the action potential into the nerve terminal (n.t.p.). This effect was readily reversible; the e.p.p. reappearing within 5 min after returning the preparation to normal Ca Ringer (Figure 1a, lower trace). The elimination of the e.p.p. in Ba Ringer was not due to an effect of Ba on the ACh-sensitivity of the subsynaptic membrane as m.e.p.ps could be readily observed (Figure 1b) despite the low (40 to 65 mV) resting potential of Ba-bathed muscle (see also Laskowski & Thies, 1972). The addition of Ba to curarized preparations bathed in Ca solutions caused a presynaptic depression of the e.p.p. amplitude which was surmountable by increasing the Ca concentration, suggesting a possible competitive relationship between the two ions. It thus appears that Ba may be an inhibitor of the process by which the action potential causes the synchronous discharge of ACh quanta detectable electrophysiologically as the e.p.p.

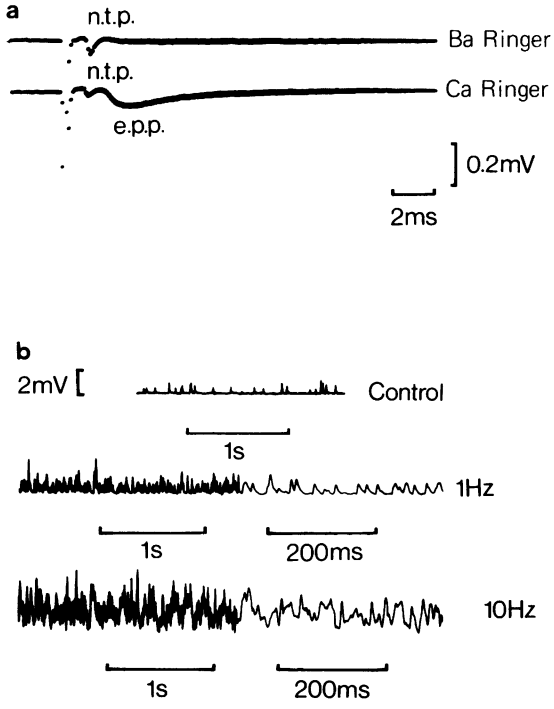

Figure 1 Effects of Ba on transmission from nerve to muscle. (a) Upper trace – absence of focal end-plate potentials (e.p.ps) after 10 min in Ba Ringer. Identical records were obtained with 1 Hz and 10 Hz stimulation. Lower trace–return of focal e.p.p. (10 Hz stimulation) after 5 min in normal Ca Ringer. n.t.p.-nerve terminal action potential. Each trace represents the averaged response to 128 stimuli. (b) All three traces are from same cell bathed in Ba Ringer with neostigmine. Upper trace – control miniature end-plate potential (m.e.p.p.) frequency (11/s). Middle trace – steady state m.e.p.p. frequency (70-75/s) attained within 1 min after beginning of 1 Hz stimulation. Lower trace – steady state m.e.p.p. frequency (»100/s) attained within 2 s after beginning 10 Hz stimulation. All 3 traces are photographs of pen records in which stimulus artifacts have been retouched for clarity. Resting potential was 46 mV. Note increased recorder speed for second half of both middle and lower traces.

It should be mentioned that although the disappearance of e.p.ps was a consistent phenomenon, the time course of this effect was quite variable. Generally e.p.ps were eliminated by 60–120 min in Ba but some cells still produced very small e.p.ps for many hours. This variability may be caused by the persistence of small amounts of calcium near certain nerve endings.

If Ba is an antagonist of synchronous quantal release, what then is the source of ACh detected in the perfusion fluid of stimulated cholinergic nerve endings? Although release of ACh from a non-quantal pool cannot be excluded, Figure 1b illustrates a possible electrophysiological correlate for the evoked ACh release, namely an asynchronous residual discharge of m.e.p.ps induced by repetitive nerve stimulation but not phase-locked to the nerve impulse (Braun, Schmidt & Zimmermann, 1966; Miledi & Thies, 1971; Hurlbut, Longnecker & Mauro, 1971). In the experiment illustrated in Figure 1b, the control m.e.p.p. frequency of 11/s (upper record) is increased to a steady-state level of between 70/s and 75/s (middle record) after 1 min of stimulation at 1 Hz and remained at that level for the duration of stimulation (5–6 minutes). The steady-state increase at 1 Hz stimulation ranged from 5 to 20 times the control m.e.p.p. frequency (7 experiments). The maximum evoked frequency often lasted for up to 5–10 s after the stimulation period was terminated. Stimulation at 10 Hz produced a rapid, intense rise of the m.e.p.p. frequency to a maintained level which could not be measured accurately (»100/s; Figure 1b, lower record). An estimate of this frequency by the method of Katz & Miledi (1972) suggests it to be »500/second. Rapid unmeasurable increases in m.e.p.p. frequencies were also produced by 10 Hz stimulation after prolonged (6 h) incubation in Ba-EGTA Ringer.

Although increases in m.e.p.p. frequencies are produced by stimulation in Ca solutions, rapid dramatic effects such as those shown in the bottom record of Figure 1b, are not seen at comparable rates of stimulation in Ca solutions (unpublished results; see also Braun *et al.*, 1966, Figure 6). It thus appears that Ba may act directly as an agonist for the asynchronous release of transmitter quanta by repetitive nerve stimulation.

Discussion The results suggest that Ba can both antagonize phasic, synchronous ACh release and, in addition, support the process of asynchronous evoked ACh release. The 5–20 fold increase in m.e.p.p. discharges detected in this study under conditions similar to those in the experiments of Douglas *et al.*, (1961) on the superior cervical ganglion (equimolar substitution of Ba for the normal ambient Ca and stimulation at 1 Hz) represent the absolute release of 13 ± 7.75 (mean $\pm$ s.e. mean, $n = 7$) *additional* quanta per impulse. Each preganglionic axon in the superior cervical ganglion normally releases «10 additional quanta per impulse when stimulated at 1 Hz in Ca solutions (Sacchi & Perri, 1973; McLachlan, 1975). It is thus suggested that the asynchronous evoked discharge of m.e.p.ps seen here in Ba Ringer is the electrophysiological correlate of the maintained evoked ACh overflow produced upon substitution of Ba for Ca at the superior cervical ganglion. In this regard similar asynchronous evoked quantal release has been observed at the guinea-pig superior cervical

ganglion bathed in Ba solutions by E. M. McLachlan (personal communication), adding considerable support to this contention.

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