The topography of posttetanic potentiation in guinea-pig ileum[1]

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Summary. The myenteric plexus-longitudinal muscle preparation of the guinea-pig ileum offers, by its anatomical arrangement, the possibility of studying a new aspect of posttetanic potentiation (PTP); its topography. Evidence was sought and obtained that during PTP more distal junctional sites of cholinergic nerve terminals may be recruited into the transmitter secretion process.

Posttetanic potentiation (PTP) at some CNS synapses has been proposed as a basic electrophysiological model of synaptic plasticity, related to the processes of learning and memory[2,3]. Until now, several explanations for the mechanism of PTP at cholinergic synapses have been suggested; the broadening of presynaptic nerve action potential due to posttetanic hyperpolarization and/or increased activity of the sodium pump; or a residual increase of intraterminal free calcium[4]. PTP has also been observed and studied at the muscarinic junction of the myenteric plexus-longitudinal muscle of the guinea-pig ileum[5,6]. Cholinergic neurons project their axons many mm aborally, run in parallel to the longitudinal muscle and have a 'boutons-en-passant' arrangement of the transmitter containing varicosities[7-9]. It is at these varicosities[10,11] that the nerve action potential may fail to invade the distal part of the terminal, or where its propagation may be facilitated during PTP; also, the recruitment of the more aborally situated neurons to participate in cholinergic transmission should be considered[11].

Materials and methods. Contractions: The myenteric plexus-longitudinal muscle preparation of the guinea-pig ileum, formed into a V-shape (fig. 1a) was placed in a bath. The 2 segments of the preparation were moistened by Vane's superfusion technique[12] so that separate electrical neurogenic stimulation of the oral or aboral segments was possible and the respective contractions were recorded. With the upper electrode attached to the oral segment, the stimulus intensity (about 5 V) was first adjusted at low frequencies (0.5 ms pulses at 0.04 Hz) so that oral segment contractions were almost maximal whereas those of the aboral segment were just suprathreshold as it was stimulated only marginally because of current spread. To prevent posttetanic twitch-inhibition due to the release of endogenous opiate ligands[13], and to diminish pretetanic twitch height, naloxone (0.5 $\mu mol \cdot l^{-1}$) and indomethacin (5 $\mu mol \cdot l^{-1}$) were added to the Krebs solution[4]; under these conditions tetanic stimulation (30 Hz, 25 sec) was applied and PTP of twitch contractions appeared. The magnitude of PTP was evaluated from the 5 min periods preceding and following tetanus where the sums of twitch amplitudes were calculated (ΣpreT and ΣpostT, respectively); the precentage change induced by tetanus was estimated as $[(\Sigma postT - \Sigma preT)/\Sigma preT] \times 100$.

EMG: A 6 cm long piece of the whole ileum was used and focally stimulated near its oral end (fig. 2a). EMG of the longitudinal muscle layer was picked up with 2 glass suction electrodes, one approximately 2–5 and the other 7–12 mm aborally from the stimulation electrode site. The recording started 10 min after mounting the preparation in Krebs solution containing naloxone and indomethacin and lasted only until spontaneous myogenic activity set in.

Results and discussion. Contractions: In 10 experiments the PTP was significantly ($p < 0.05$; t-test for paired data) more pronounced in the aboral segment (324 ± 102%) than in the oral segment (42 ± 19%; fig. 1b). This might be due to the possibility that cholinergic terminals projecting aborally are recruited during PTP; the aborally extending zone of excitation is thus located mainly in the aboral segment and may substantially contribute to its contractions. On the other hand, smaller PTP in the oral segment may be due to greater intensity of the stimulatory current exciting most nerve terminals present at many points[14] and leaving less room for varicosity or neuron recruitment. Apart from the recruitment process, other mechanisms of PTP[15] could also contribute to larger potentiation of suprathreshold twitches. However, only the former mechanism should be strictly dependent on the oral-aboral orientation of the V-shaped preparation whereas the latter mechanism might pertain only to twitch amplitude before tetanus[6].

To test this possibility, the upper electrode was placed on the aboral segment and its maximal contractions were seen in the oral segment (fig. 1c). Under these conditions PTP in the oral segment (86 ± 51%; 10 exps) did not significantly exceed that in the aboral segment (71 ± 31%). Thus, large PTP in the aboral segment during oral segment stimulation was not solely due to low twitch amplitude there, but rather due to that orientation of the preparation which provided better opportunity for recruitment, as cholinergic terminals project mainly aborally. The possibility that not just distal varicosities within one neuron but more cholinergic interneurons in the aboral segment were recruited during PTP was ruled out by the observation that d-Tubocurarine (130 $\mu mol \cdot l^{-1}$; 4 exps) did not prevent the much larger PTP in the aboral segment. The possibility that more cholinergic neurons were recruited there by other interneurons which utilize e.g. peptides as their neurotransmitters, remains open[11].

EMG: With low stimulus voltage (10–30 V) the neurogenic response (N-EMG) was usually present at the oral site but absent at the aboral site (fig. 2). The latency of N-EMG after stimulation artifact was 287 ± 30 msec in 11 experiments. A train of 3–10 pulses (0.2 msec; 100 Hz) was usually necessary

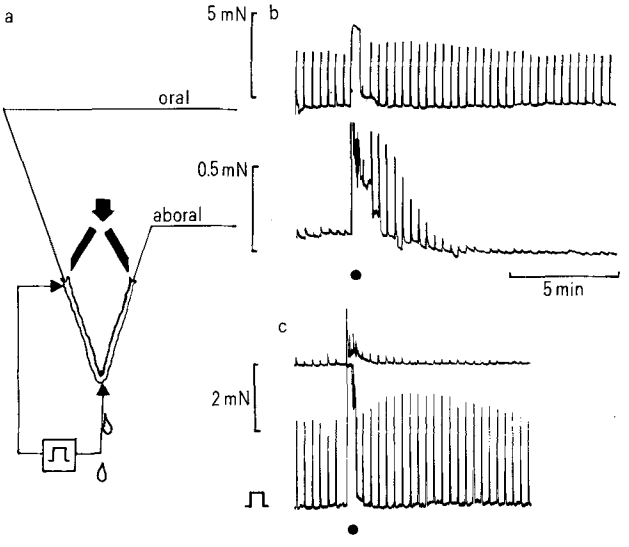

Figure 1. The contractions of the V-shaped preparation. *a* Experimental arrangement. *b* The record of a typical experiment where stimulation electrodes were placed as shown in (a). Twitch contractions following tetanus (●) were distinctly potentiated only in the aboral segment. *c* The record of an experiment where the stimulation electrodes were placed on the aboral segment (_⊓⊔_); following tetanus twitch contractions of both segments were potentiated but to a similarly small degree.

to check whether the N-EMG response could be picked up at the aboral electrode. The stimulus bound N-EMG could be differentiated from the secondary excitation of the muscle layer that was activated by the passage of the propagated muscle action potential set up in other regions. This secondary excitation was usually observed only at the aboral electrode site and was loosely related to the N-EMG seen at the oral electrode site (figs. 2b, d, e). The N-EMG at both sites completely disappeared in the presence of tetrodotoxin (1 μmol·l^{-1}; 5 exps) but was unaffected by d-Tubocurarine (130 μmol·l^{-1}; 7 exps). In 8 experiments the N-EMG was tested 12 times before tetanus, and was present in 94 ± 3 and 11 ± 5% at the oral and aboral sites, respectively. In the interval of 40–150 sec after tetanus (30 Hz, 15 sec) the incidence of N-EMG did not change at the oral site (96 ± 2%) but increased to 71 ± 9% ($p < 0.005$, t-test for unpaired data) at the aboral site. The latter value decreased in the subsequent interval (160–270 sec) to 37 ± 15%. The increased occurence of N-EMG responses at the aboral site in the first posttetanic interval coincided with the time course of twitch potentiation (cf. fig. 1b). These experiments showed that during PTP the N-EMG responses were initiated several mm more aborally, at a site where they were absent before. Since N-EMG response was considered to be a direct consequence of the localized action of the released transmitter, its presence indicated that the more distal varicosities of the same nerve terminal or more distal nerve terminals of other neurons had been recruited.

The process of nerve action potential invasion into the distal part of its varicose terminal has not yet been directly demonstrated in a mammalian preparation[16,17]. Because of the ar-

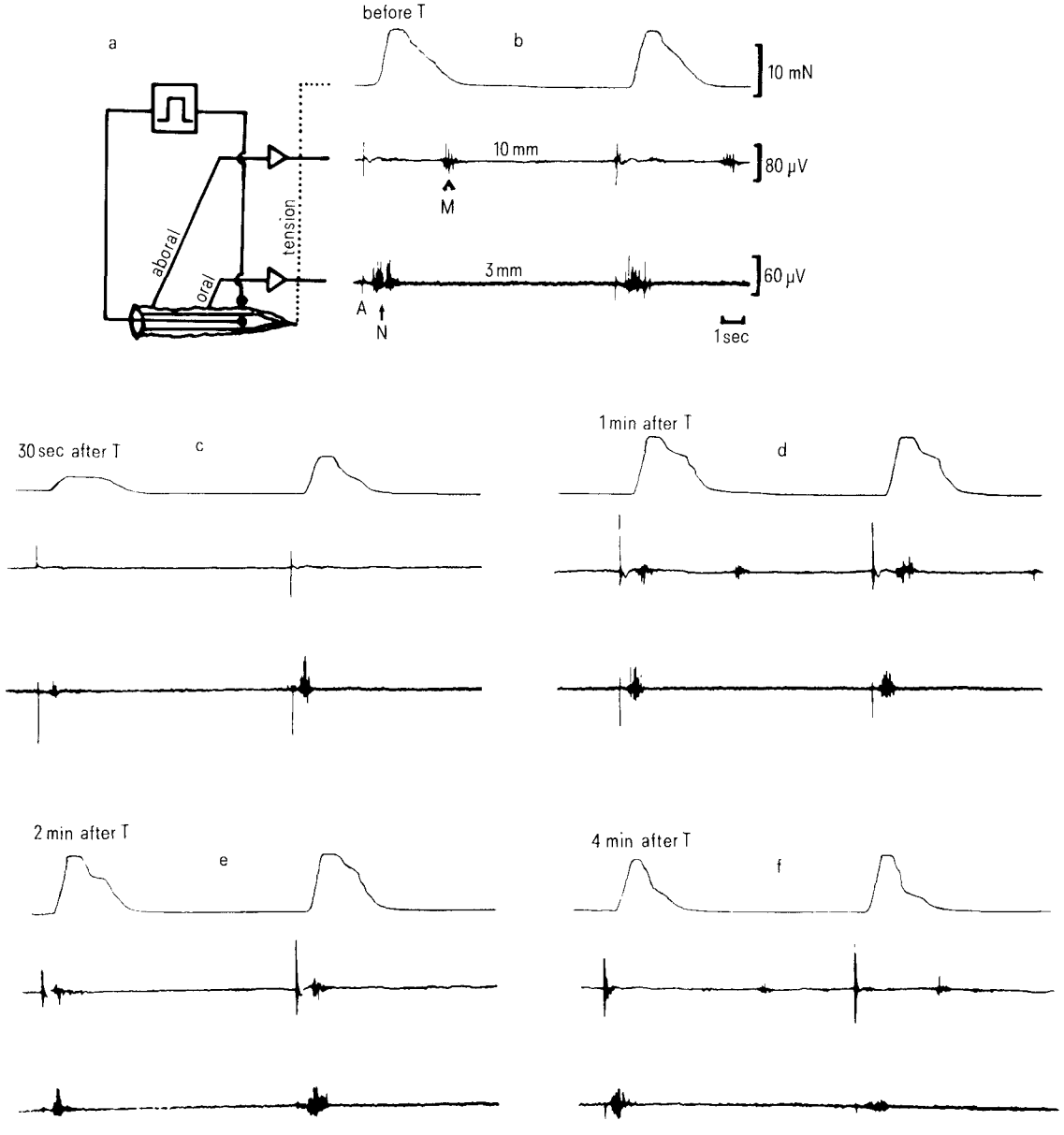

Figure 2. The EMG record of the longitudinal muscle layer. *a* The experimental arrangement shows the preparation of the whole ileum pulled onto a perspex rod with the stimulating and recording electrodes in their respective positions. *b–f* The record of a typical experiment starting 2 min before (b) tetanic stimulation (T); single pulses (10 V) evoked stimulation artifact (A) followed by neurogenic EMG response (N) observed at the site of oral electrode (3 mm, lower trace) throughout the experiment (b–f); at the site of aboral electrode (10 mm, middle trace) the N-EMG response was seen only 1 and 2 min after tetanus (d and e, respectively); in some other cases (b, d, f) secondary or myogenic excitation (M) was observed several seconds after stimulation. Contractions are recorded in the upper trace.

rangement of the myenteric plexus as it has been described so far[11], comprising cholinergic, peptidergic and other neurones, the approaches used in this study have not allowed us to distinguish whether only cholinergic neurones participate in the phenomenon of PTP. However, the hypothetical recruitment of more distal sites of cholinergic transmission described here stresses the role played by response topography in the mechanism of synaptic plasticity during PTP.

1 Acknowledgments. We thank Prof. G. Burnstock, Drs J. Bureš and V. Bauer for helpful criticism on the manuscript and Mr V. Růžička for expert assistance.
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0014-4754/84/040404-03$1.50 + 0.20/0

Degradation of chlorobenzoates by *Aspergillus niger*

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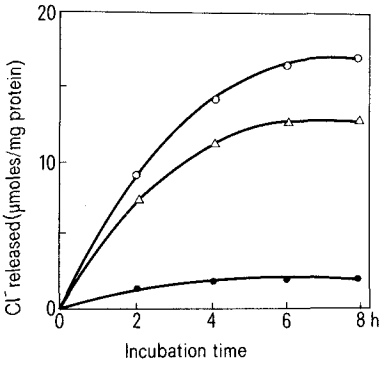
Summary. In *A. niger*, the degradation of chlorobenzoates follows the protocatechuate branch of β-ketoadipate pathway and the elimination of chloride takes place in the first hydroxylation step prior to ring cleavage.

The ability of microorganisms to dehalogenate chlorinated molecules is of great practical importance, because many of these chemicals are widely used and released into water and soil. Chlorobenzoates are a group of compounds that occur in the environment in large amount either because of their release as herbicides[2], or as products of cometabolism of polychlorinated biphenyls by mixed or pure cultures[3]. Without exception, these haloaromatic compounds are markedly more refractory to microbial attack than non-halogenated aromatics. In the catabolic pathways so far described, no enzymic activity has been found for the direct hydrolytic cleavage of the halo-arene bond[4]. The present paper describes the degradation and dehalogenation of chlorobenzoates by *Aspergillus niger*.

The strain of *A. niger* used in the present studies was isolated from a complex petrochemical sludge, as described earlier[5]. The maintenance and cultivation conditions of this strain were the same as described earlier[6,7]. *A. niger* was grown in shake culture at 28 ± 1 °C using chlorobenzoates, benzoate (0.25% w/v) or glucose (2% w/v) as carbon sources in a synthetic medium[5]. Oxygen uptake was measured by using washed cell suspension, as described earlier[6]. The methods used for the isolation and identification of metabolites were the same as described earlier[6,7]. The preparation of cell-free extracts and methods for enzymatic analysis were the same as described previously[6,8].

Dehalogenation by cells. The 48-h-grown cells were harvested and washed twice with sterile distilled water. The cells were resuspended (ca 5 mg dry weight) in 50 ml of 0.1 M Tris-SO_4 buffer (pH 7.6) containing 0.1 M 2-chlorobenzoate (2-cba) or 3-chlorobenzoate (3-cba), respectively. The filter-sterilized stock solution of cycloheximide was added at a final concentration of 2 mg/ml. After incubation for 12 h, the chloride concentration was determined using uninoculated blanks as control.

The dehalogenating activity of the cell-free extracts was measured by determining the amount of chloride initially present in the reaction mixture. The reaction mixture contained: Tris-SO_4 buffer (pH 7.6) 50 mM, 2-cba 2.5 mM, NADPH 1 mM, FAD 1 μM and 2-3 mg of cell-free extract protein to a final volume of 20 ml. The liberation of chloride released was determined by using an ion-sensitive chloride electrode[8]. The dehalogenating activity of cell-free extracts in the absence either of NADPH or of FAD was significantly reduced, and it was also dependent on the amount of protein added to the reaction mixture. The reaction product was identified by using methods described previously[7]. Protein was determined by the method of Lowry et al.[9].



The time course of dehalogenation of 2-cba by cell free extracts of *A. niger* cells grown on 2-cba (○—○), benzoate (△—△) and glucose (●—●).