after 6 h activity has fallen by only some 16% and after 24 h there is still some 25% residual activity. If the solution is maintained at 4 °C there is no measurable loss of activity.

The loss of activity is not restricted to 5-HTCS in solution: Figure 3 shows that if solid 5-HTCS is kept at room temperature then the stimulatory 5-hydroxytryptamine activity is markedly reduced after 84 days (3 months) and is abolished after 140 days (5 months); even if the sample is maintained dehydrated at −20 °C activity is reduced to some 75% of its original after 224 days (8 months). Creatinine sulphate activity does not appear to be seriously impaired by keeping the solid 5-HTCS at room temperature. Preliminary investigations have shown that the potency[9] of 5-HTCS is unaffected by any of the above treatments; the loss of 5-hydroxytryptamine activity in stimulating sodium transport thus probably reflects the gradual decomposition of the compound to produce an inactive derivative. This being the case the process is not photo-chemical since, as shown in the Table, maintaining 5-HTCS (either solid or in solution) in darkness does not prevent the decline in activity.

The relative activities of solutions and solid samples of 5-HTCS maintained at room temperature but under different conditions of light exposure

Solution (5 h)		Solid samples (4 month)	
Light	Dark	Light	Dark
0.21	0.26	0.44	0.51

In all cases the figures quoted are the relative activity of a 10^{-5} *M* response and represent the mean of 4 experiments.

A similar decrease in activity of 5-hydroxytryptamine has been observed by BERRIDGE (personal communication) in the stimulation of fluid secretion by the isolated salivary glands of *Calliphora erythrocephala* and by CALVERT (personal communication) in the stimulation of fluid secretion by the salivary glands of *Musca domestica*. Such changes in the activity of 5-HTCS solutions reflect either the specificity of the tissue receptor or the ability of the receptor-hormone complex to initiate the physiological response. MADDRELL et al.[10] and BERRIDGE[11] have shown that small changes in the 5-hydroxytryptamine molecule, particularly with respect to the hydroxyl group at C_5 and the quaternary nitrogen on the end of the ethylamine side chain, leads to an inactivation of the molecule but does not necessarily prevent its binding. It seems probable therefore, that the loss of 5-hydroxytryptamine activity is due not to the inability of the derivative to bind to the tissue receptor but rather to the inability of the receptor-derivative complex to initiate the physiological response. The present work thus indicates that tissues may appear to be falsely unresponsive to applied 5-hydroxytryptamine if either the solid or the prepared solution is allowed to stand at room temperature; indeed if a tissue exhibits both a stimulatory 5-hydroxytryptamine response and an inhibitory creatinine sulphate response then because of the loss of activity of 5-hydroxytryptamine but not of creatinine sulphate to the same degree the tissue could appear to be inhibited (creatinine sulphate response) by 5-hydroxytryptamine.

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Depletion of Synaptic Vesicles at the Frog (*Rana pipiens*) Neuromuscular Junctions by Tetraphenylboron

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Summary. At the frog neuromuscular junction tetraphenylboron produced a decrease in miniature endplate potential amplitude, accompanied by a decrease in the number but not the size of synaptic vesicles.

Tetraphenylborate (TPB), a highly lipid soluble anion, exhibits marked pharmacological effects at the amphibian neuromuscular junction[3,4]. Initially TPB produces a profound concentration-dependent increase in spontaneous acetylcholine release and augments evoked release. With time the high rate of spontaneous release declines and this decline is accompanied by a decrease in quantal size. Further, neuromuscular transmission is blocked by TPB, presumably by a pre-junctional mechanism, as TPB is devoid of post-junctional agonist or antagonist properties[3,4].

The present study represents an attempt to correlate the TPB-induced changes in the rate of spontaneous transmitter release and of quantal size measurable by electrophysiological techniques, with morphological changes at the frog neuromuscular junction. In particular, it was of interest to observe whether depletion of synaptic vesicles occurred and whether the reduction of quantal size was accompanied by a decrease of synaptic vesicle diameter, as such a correlation is not noted after quantal size reduction by hemicholinium[5,6].

For electrophysiological recordings, frog (*Rana pipiens*) sartorius muscle preparations were dissected in a phosphate buffered Ringer solution[7] and were subsequently

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maintained at room temperature (16–20 °C), in a K^+-deficient *Tris*-buffered Ringer solution of the following composition: NaCl 120 m*M*; $CaCl_2$ 1.8 m*M*; *Tris* (*tris*-(hydroxymethyl)-aminomethane) 1 m*M*. Previous experiments[3,4] were performed in K^+-containing solutions, but K^+-deficient solutions were used in these experiments to avoid precipitation of the water insoluble potassium salt of TPB, and to afford comparison with the experimental conditions used in the electron microscope studies. The solution was made 10% hypertonic by the addition of sucrose, to elevate the resting miniature endplate potential (MEPP) frequency. For the present study a concentration of TPB (0.05 m*M*), as the sodium salt, was selected that is capable of producing neuromuscular block of surface muscle fibres within 30 min and that is lower than that which produces an explosive increase in MEPP frequency and which also blocks the nerve terminal spike[3,4]. TPB (0.05 m*M*) was dissolved in an identical solution to that bathing the muscle. TPB was applied to individual motor endplates by local microperfusion[8]. Endplates were visualized by light microscopy under 300 × magnification so that electrode impalement and drug microperfusion could be performed under the same magnification. Intracellular recordings of MEPP were made using standard electrophysiological techniques. KCl-filled glass microelectrodes (3 *M* KCl, 6–10 *MΩ*

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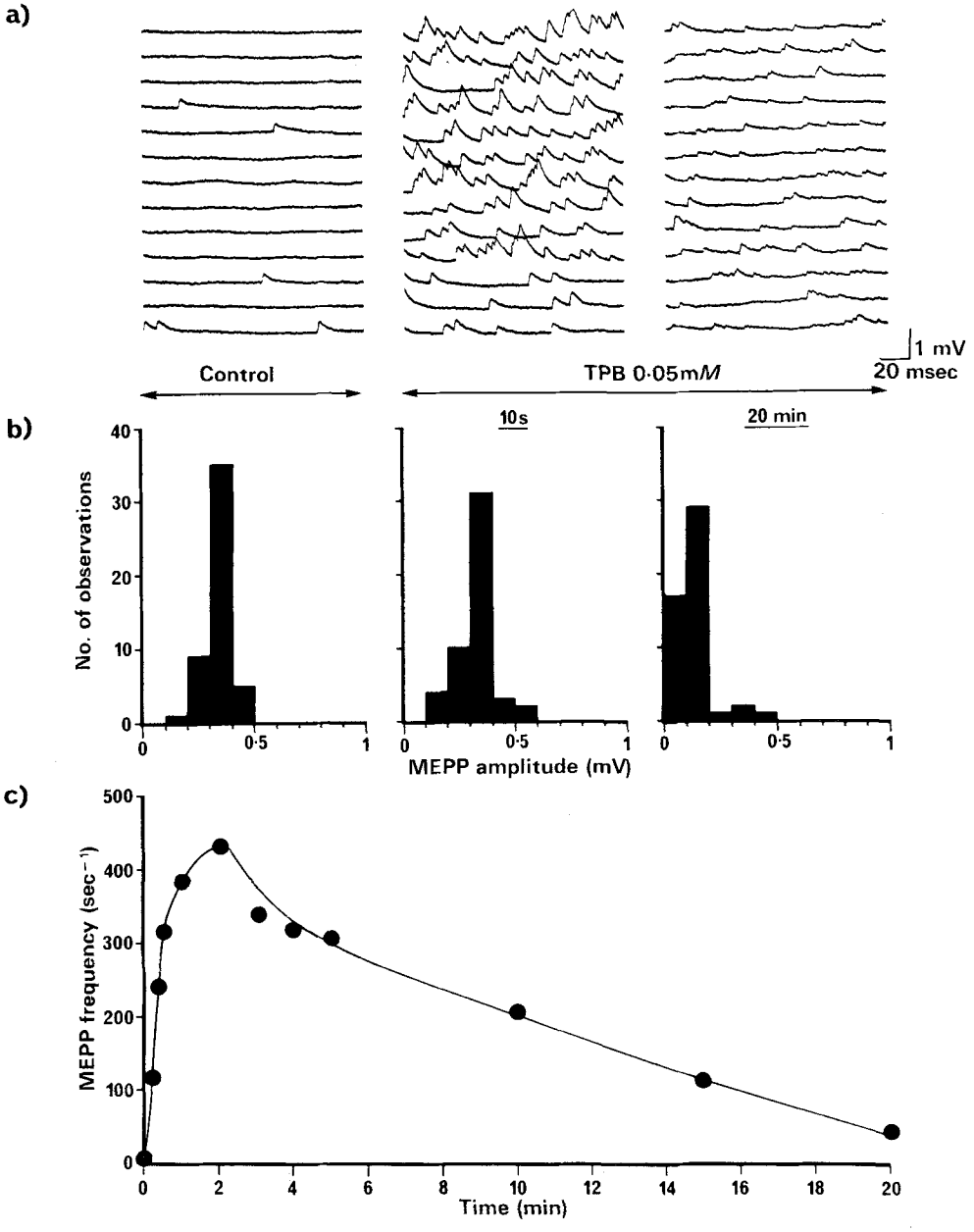

Fig. 1. Effects of local microperfusion of TPB (0.05 m*M*) on spontaneous transmitter release at an individual frog motor endplate. a) Intracellular recording of miniature endplate potential (MEPP) activity before, immediately after the commencement and after 20 min of TPB microperfusion. b) MEPP amplitude distribution histograms for the periods illustrated in a). c) MEPP frequency during microperfusion of TPB.

resistance) were used to record membrane potentials and MEPP. Membrane potentials were standardized at −90 mV by passing DC current through a second microelectrode inserted in the endplate region of the muscle fibre.

For electron microscopy whole sartorius muscles were dissected from frogs and were pinned on Sylgard-coated dishes at normal resting tension. Muscles were exposed to TPB (0.05 m*M*) dissolved in K^+-deficient Ringer solution for a period of 30 min. Control muscles were bathed in K^+-deficient Ringer solution for 30 min. The tissues were fixed in a 0.1 *M* sodium cacodylate buffered 2% glutaraldehyde-2% paraformaldehyde solution containing 10 m*M* $CaCl_2$ for 4 h. The muscles were then rinsed in the buffer for 30 min and lightly osmicated (1% osmium tetroxide) for 5 min to facilitate visualization of the nerve branches. Small triangles of tissue containing nerve terminals at their apexes were dissected from edge regions of the preparation where the muscle is only a few fibres thick. Dissection was performed whilst the tissue was bathed in the buffer solution. The segments of tissue were postfixed for 2 h in 0.1 *M* cacodylate buffered 1% osmium tetroxide and then rinsed several times in buffer. All fixing solutions contained 10 m*M* $CaCl_2$. The tissue was dehydrated in a graded ethanol series, passed through propylene oxide and embedded in Epon 7[9].

Thick (1 μm) sections were taken along both the transverse and longitudinal axes of the fibres until neuromuscular junctions were encountered. Thin (60 nm) sections were cut, mounted on copper grids, stained with uranyl acetate, and examined with a Philips 300 electron microscope.

Synaptic vesicles were counted and measured manually from electron micrographs using a Bausch & Lomb dissecting microscope with an eyepiece graticule. Mean synaptic vesicle diameter was obtained from measurements of 20 vesicles selected at random in each nerve terminal section.

Statistical analyses were performed by Student's *t*-test, *p*-values of less than 0.05 being regarded as significant.

As previously reported[3,4] locally perfused TPB (0.05 m*M*) initially produced a large increase in MEPP frequency. Peak frequency was attained within the first 2 min of drug application, and was followed by a gradual decrease in frequency over a 30 min period of exposure (Figure 1a and c). During the initial increase in MEPP frequency no change in MEPP amplitude occurred (Figure 1b). No measurement of MEPP amplitude was attempted at the peak of MEPP frequency as, at the high frequencies observed, summation of MEPP occurred. However, after the peak, a marked reduction of measurable MEPP amplitude was observed. For example, in the results illustrated in Figure 1a and c in 1 fibre mean measurable MEPP amplitude was reduced by 54% after 20 min exposure to 0.05 m*M* TPB. After this time MEPP amplitude fell to the extent that it was no longer possible to distinguish MEPP from noise and hence no reliable measurement could be made after approximately 20–25 min.

After 30 min of TPB treatment (Figure 2b) vesicle numbers per unit area of nerve terminal transverse sections were significantly reduced ($p < 0.05$) from the control (Figure 2a) value of 140 ± 11 vesicles/μm² (mean ± SEM; $n = 6$) to 49 ± 11 vesicles/μm² ($n = 3$). There was no significant change ($p > 0.05$) in vesicle diameter after 30 min of TPB treatment. The mean vesicle dia-

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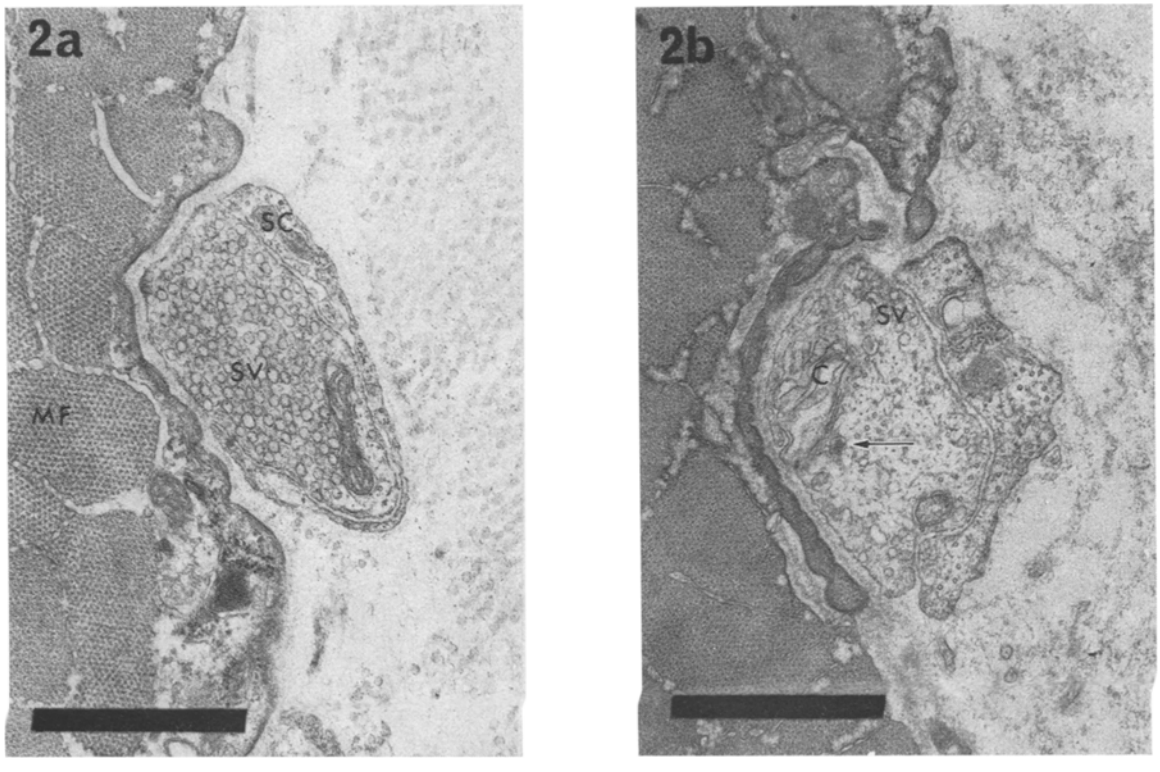

Fig. 2. Ultrastructural analysis of nerve terminals following a treatment with 0.05 m*M* TPB. Calibration bars = 1 μm. a) Transverse section of a nerve terminal in a control preparation. SV, synaptic vesicles; SC, Schwann cell cytoplasm; MF, muscle fibril. × 27,700 b) Transverse section of a nerve terminal 30 minutes following TPB administration. A few synaptic vesicles (SV) are present. Also contained within the ending are cisternae (C) and an occasional dense-core vesicle (arrow). × 27,700.

meter in 5 untreated terminals was 535 ± 11 Å, whereas in muscles treated with TPB for 30 min the mean vesicle diameter was 545 ± 4 Å ($n = 3$). In the TPB-treated preparations the remaining vesicles tended to be clustered at the presynaptic membrane and occasionally fusion vesicles were observed. Similar observations were made in longitudinal sections from the same muscle preparations.

This pattern of partial depletion, which probably indicates that vesicle recycling[10] is proceeding normally, has also been reported after procedures that greatly increase transmitter release e.g. rapid nerve stimulation[10,11] and lanthanum treatment[12]. However the pattern of vesicle depletion produced with this concentration of TPB differs from the total depletion produced by black widow spider venom[13,14], although higher concentrations of TPB may produce more drastic depletion. Greatly increased transmitter release has been shown recently to lead to a predominance of small amplitude MEPP[15,16]. Consequently, the marked increase of MEPP frequency in TPB may be a factor contributing to the reduction of quantal size noted. Other possible explanations for this reduction are that TPB may reduce the concentration of acetylcholine in the vesicles by precipitation or that TPB inhibits transmitter storage. A reduction of quantal size is seen after prolonged nerve stimulation in the presence of hemicholinium-3[17], although TPB itself appears to have no inhibitory action on choline uptake[4] or choline acetyltransferase[18]. In agreement with recent findings with hemicholinium in the frog[5,6] it was found that TPB produced no reduction of vesicle size, indicating that drug-induced reduction of quantal size is not accompanied by a reduction of vesicle diameter and that the vesicles observed after either TPB or hemicholinium treatment are either empty or only partially filled with acetylcholine.

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Does an Initial Phasic Response Exist in the Receptor Potential of Taste Cells?

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Summary. The depolarizing receptor potentials to 0.5 *M* NaCl recorded from frog taste cells did not exhibit any phasic response, even when the rectangular waveform of stimulus onset was employed. The quickest depolarizations recorded reached the peak in 50 msec. On the other hand, the gustatory neural response showed initial overshoot of the impulse discharge even when 0.5 *M* NaCl was delivered at the slower rate of 0.06 ml/sec. It is concluded that the initial neural response may be associated with the rate of rise of the receptor potential before its plateau level is reached.

Depolarizing receptor potentials, which may be associated with the initiation of gustatory neural impulses, have been recorded intracellularly from vertebrate taste cells[2-8]. The depolarizations indicate a sustained response having no initial overshoot.

On the other hand, gustatory neural activities to NaCl, as well as other salts, usually consist of an initial phasic response followed by a smaller tonic response[9]. Because of lack of the initial phasic response in the taste receptor potentials, SATO and BEIDLER[8] have proposed that the initial phasic component of the gustatory neural responses may be related to the rate of rise of the receptor potentials or the following postsynaptic potentials. However, recently it has been suggested that since a very slow rate of taste stimulus onset was employed for the microelectrode study on taste cells, the initial phasic response in the taste receptor potential, which may be correlated with the phasic discharge in the gustatory nerve, could not have been found even if present[10,11].

Therefore, the purpose of the present experiments is to see whether or not the taste receptor potentials possess an initial overshoot component when using the rapid rate of rise of a taste stimulus. It will be shown that the taste receptor potentials having a 50 msec rise time still do not exhibit any overshoot component. Thus, it is suggested that the initial phasic burst of activity in the gustatory nerve may be related to the rate of rise of taste receptor potential.

Materials and methods. Tongues of bullfrogs (*Rana catesbeiana*) anesthetized with urethane were used in this experiment. The 3 *M* KCl-filled glass capillary microelectrodes of 15–45 MΩ were inserted into taste cells of the fungiform papillae. An indifferent electrode of chlorided silver wire was put into the musculature of a forelimb.

[1] I thank Professor M. ICHIOKA for his encouragement during the work and Miss M. OGURO and Mrs. K. SUGIMOTO for their valuable help in preparing the manuscript. Thanks are also due to Dr. D. TUCKER for his critical reading of the manuscript. This work was supported in part by a grant (No. 57098) from the Ministry of Education in Japan.
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