Br. J. Pharmac. (1980), **69**, 249–255

POST-TETANIC POTENTIATION IN GANGLIA WHICH ARE BLOCKED WITH HEXAMETHONIUM

DARYL CHRIST

Department of Pharmacology, University of Arkansas for Medical Sciences, Little Rock, Arkansas 72201, U.S.A.

1 Post-tetanic potentiation (p.t.p.) of the compound action potential in the presence of hexamethonium was observed in the isolated stellate ganglion of the hamster using extracellular postganglionic recordings.

2 The magnitude of the p.t.p. was small (less than a 20% increase) in the control solution, but increased as the depth of blockade with hexamethonium was increased.

3 The magnitude of the p.t.p. was frequency-dependent between 1 and 40 Hz.

4 Atropine partially blocked the p.t.p.

5 McN-A-343, a muscarinic agonist, potentiated ganglionic transmission which had been partially blocked by hexamethonium.

6 Repetitive stimulation in the presence of hexamethonium potentiated the discharges induced by DMPP, a nicotonic agonist. The potentiation was blocked by atropine.

7 It was concluded that the p.t.p. in the presence of hexamethonium has the same characteristics as p.t.p. in the control solution. There appears to be both a muscarinic component and a presynaptic component of the p.t.p.

Introduction

A prolonged period of post-tetanic potentiation (p.t.p.) of the evoked potential has been observed in the unblocked sympathetic ganglion (Bronk, Tower, Solandt & Larrabee, 1938; Larrabee & Bronk, 1947; Libet, 1964; Brimble, Wallis & Woodward, 1972). It appeared to have two underlying components. A p.t.p., occurring when conditioning and test pulses were applied to different preganglionic nerves (heterosynaptic stimulation), was completely blocked by atropine. However, a p.t.p., occurring when conditioning and test pulses were applied to the same preganglionic nerve (homosynaptic stimulation), was only partially blocked by atropine. On the basis of these results, Libet (1964) hypothesized that muscarinic transmission in the ganglion was partially responsible for the p.t.p. It was also suggested that the atropine-insensitive component of the p.t.p. was due to a presynaptic mechanism (Libet, 1964; Brimble & Wallis, 1974).

P.t.p. of the evoked potential has also been observed in stellate ganglia which were blocked by (+)-tubocurarine (Eccles, 1952); however, the characteristics of the p.t.p. in the presence of a nicotinic competitive blocking drug have not been explored to determine if the p.t.p. is generated by the same basic mechanisms as in unblocked ganglia. The objective of this paper is to describe the characteristics of p.t.p. in the presence of hexamethonium, a ganglionic blocking drug. A preliminary report of these findings has been published (Christ, 1977a).

Methods

All experiments were performed on the isolated perfused stellate ganglion of the hamster, as described previously (Christ, 1977b). The ganglion with short sections of preganglionic and postganglionic nerves, was isolated and suspended in a small chamber (volume less than 1 ml). The preganglionic and postganglionic nerves made contact with bipolar platinum electrodes in side chambers. The preganglionic nerve was stimulated with supramaximal pulses of 0.1 ms duration. Potentials from the postganglionic nerve were amplified and displayed on an oscilloscope. Permanent records were made with an oscilloscope recording camera. The amplitude of the compound action potential was determined by measuring the voltage from the baseline to the first peak.

The ganglion was superfused with a physiological solution (Liley, 1956). Choline chloride (3×10^{-5}M) was added to the solution to avoid transmitter de-

0007-1188/80/060249-07 $01.00

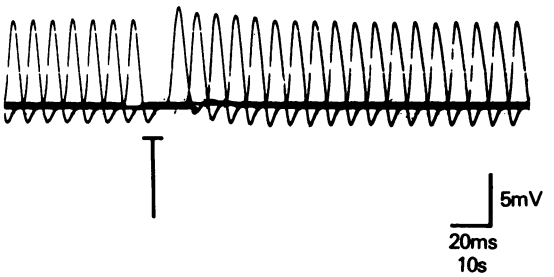

Figure 1 Post-tetanic potentiation (p.t.p.) of compound post-ganglionic action potentials elicited by preganglionic nerve stimulation of the isolated stellate ganglion of the hamster. This is a record of action potentials at 0.2 Hz from a triggered oscilloscope sweep photographed with continuously moving film. At the mark, the preganglionic nerve was stimulated at a frequency of 30 Hz for 5 s.

pletion. This concentration of choline chloride had no observable effect on ganglionic transmission at low stimulation frequencies. The solution was aerated with 95% O_2 and 5% CO_2 and maintained at a constant temperature in the range of 36° to 38°C. Drugs were applied by adding them to the reservoir of superfusing solution which flowed into the ganglion chamber.

The drugs used were: hexamethonium chloride (Nutritional Biochemicals, Cleveland, OH), atropine sulphate (Sigma Chemical Co., St. Louis, MO), 1,1-dimethyl-4-phenylpiperazinium (DMPP) (Aldrich Chemical Co., Milwaukee, WI), and 4-(*m*-chlorophenylcarbamoyloxy)-2-butynyl-trimethylammonium chloride (McN-A-343) (McNeil Laboratories, Fort Washington, PA).

Results

Post-tetanic potentiation

Compound action potentials were elicited by supramaximal stimulation of the preganglionic nerve and recorded from the postganglionic nerve. A conditioning train of stimuli (30 Hz, 5 s) to the unblocked stellate ganglion produced small increases in the amplitude of the post-train compound action potentials. Initially, the p.t.p. was determined from a single test stimulus delivered from 1 s to 5 min after a conditioning train. However, this resulted in considerable error because nearly 1 h was needed to determine the time-course of the p.t.p. In subsequent experiments, the p.t.p. was observed by superimposing a conditioning train on test pulses continuously delivered at a frequency of 0.2 Hz. At 0.2 Hz, no significant potentiation of the compound action potentials occurred. In 17 preparations which were stimulated at 30 Hz for 5 s, there was p.t.p. in 13, no change in 1, and post-tetanic depression in 3. In the ganglia with p.t.p., the average increase in amplitude of the compound action potential was 15% and the average duration of the increase was 25 s (Figure 1). No attempt was made to measure the changes which occurred within the first 5 s after the conditioning train.

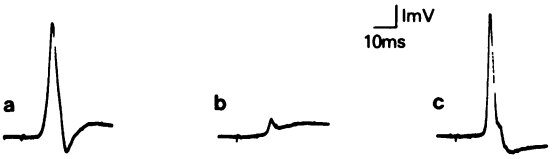

Figure 2 Post-tetanic potentiation in the presence of hexamethonium (5×10^{-4}M). Hexamethonium (b) reduced the amplitude of the action potential by 85%, as compared to the control (a). The potential was increased after repetitive stimulation at 30 Hz for 5 s in the presence of hexamethonium (c).

When the ganglia were partially blocked with hexamethonium, p.t.p. occurred in all preparations. Hexamethonium (5×10^{-4}M) reduced the amplitude of the compound action potential to an average of 19% of the control amplitude. During the period of p.t.p. produced by a conditioning train of stimuli (30 Hz, 5 s), the post-train potentials increased to pre-hexamethonium amplitudes (Figure 2). In other words, high frequency stimulation completely reversed the blocking effects of hexamethonium. The p.t.p. reached its

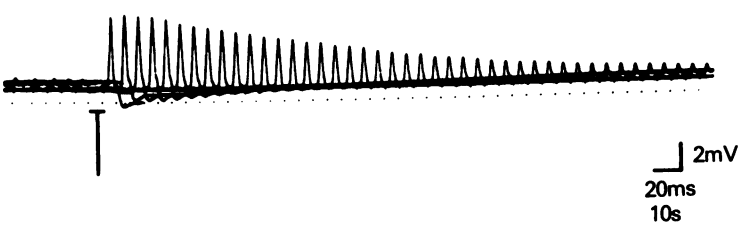

Figure 3 Time-course of post-tetanic potentiation in the presence of hexamethonium (5×10^{-4} M). Action potentials were recorded as in Figure 1. At the mark, the preganglionic nerve was stimulated at a frequency of 30 Hz for 5 s.

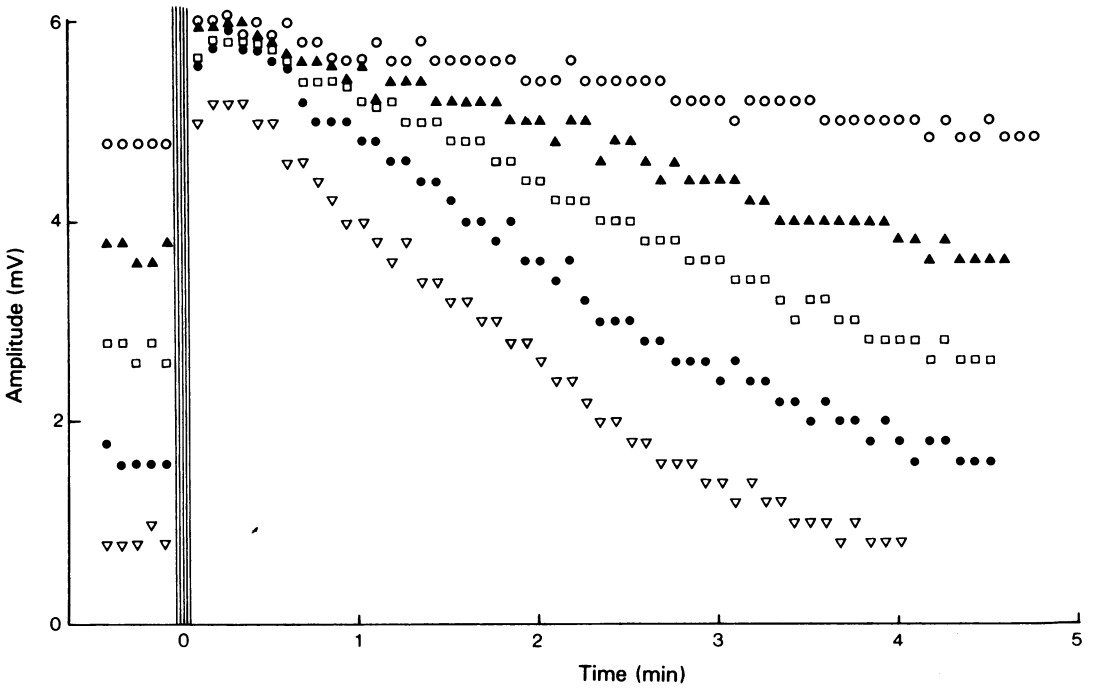

Figure 4 Post-tetanic potentiation in several concentrations of hexamethonium. The pre-train amplitude is indicated before the vertical bar. At the bar, the preganglionic nerve was stimulated at 30 Hz for 5 s. (○) = 10^{-4} M hexamethonium; (▲) = 2×10^{-4}M, (□) = 3×10^{-4}M; (●) = 4×10^{-4}M, and (▽) = 5×10^{-4}M. Each point is the amplitude of one potential, and all the results are from a single preparation.

maximum within 5 s after the train and had a duration of 3 to 5 minutes (Figure 3).

Depth of blockade

The magnitude of the p.t.p. depended upon the depth of blockade by hexamethonium (Figure 4). At 10^{-4}M hexamethonium, the ganglion was only partially blocked, yet the conditioning train increased the compound action potential to the same amplitude as when the ganglion was blocked with 4×10^{-4}M hexamethonium. This would indicate that there is a maximum potential amplitude which can be produced. The maximum may simply be due to 100% transmission. As the degree of ganglionic blockade was increased, the relative magnitude of the p.t.p. increased; but the absolute maximum did not change. With the deeper block, the p.t.p. was at the maximum amplitude for a shorter period of time, and decayed at a faster rate. In the experiment in Figure 4, the slope of the decaying phase of the p.t.p. increased as the depth of the block increased. In fact, there was a direct relationship between the depth of the blockade and the slope of the decaying phase.

Stimulation frequency

The p.t.p. was frequency-dependent. Trains with frequencies as low as 1 Hz (5 s) induced p.t.p. In the experiment in Figure 5, 1 Hz, in the presence of 5×10^{-4}M hexamethonium, caused a p.t.p. of 160%. As the frequency was increased, the magnitude and duration of the p.t.p. increased, until the frequency was at 20 or 40 Hz. The primary change at 40 Hz, as compared to 20 Hz, was an increase in the duration of the p.t.p.

Effects of atropine

Atropine altered the p.t.p., but did not abolish it. At concentrations as low as 10^{-8} M, atropine reduced the p.t.p. in 5×10^{-4} M hexamethonium (Figure 6). However, even with 10^{-6} M atropine, the magnitude of the p.t.p. was only reduced by approx. 50%. The duration was also reduced, but the time to maximum p.t.p. was increased. This indicates that a muscarinic mechanism was involved in the generation of the p.t.p., but there must also be a non-muscarinic mechanism.

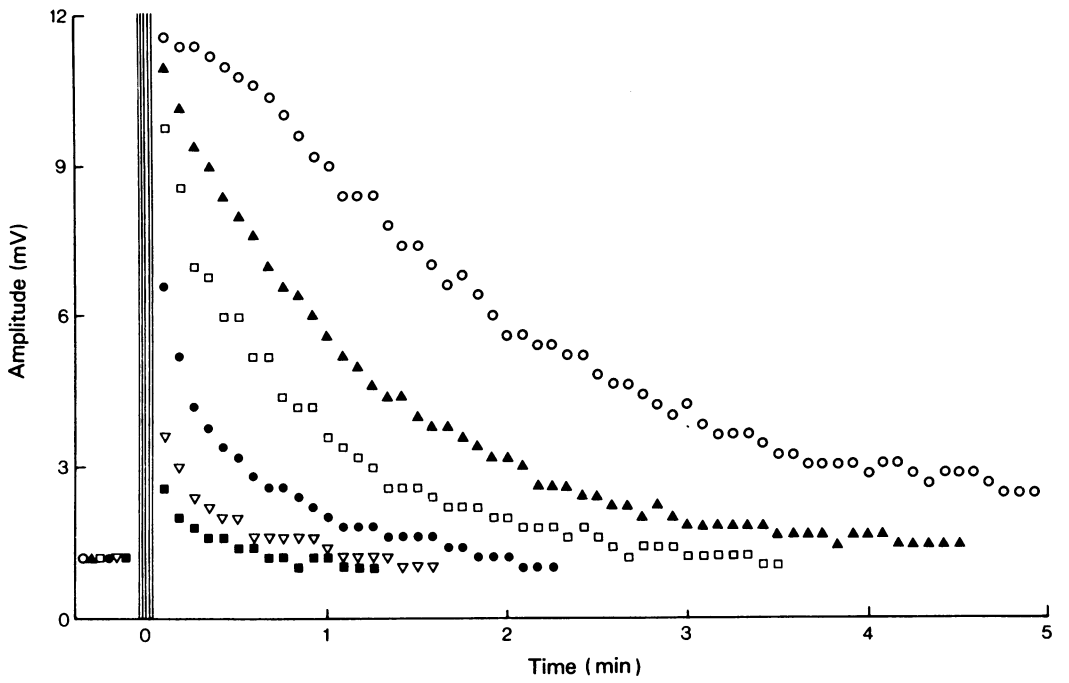

Figure 5 Post-tetanic potentiation from several frequencies of preganglionic nerve stimulation in 5×10^{-4}M hexamethonium. At the bar, the preganglionic nerve was stimulated for 5 s at: (■) = 1 Hz, (▽) = 2 Hz, (●) = 4 Hz, (□) = 10 Hz, (▲) = 20 Hz, and (○) = 40 Hz. Each point represents the amplitude of one potential and all the results are from a single preparation.

It was observed that increasing the concentration of atropine from 10^{-7}M to 10^{-6}M often increased the p.t.p. In other words, the p.t.p. in 10^{-6}M atropine was larger than the p.t.p. in 10^{-7}M atropine. This property of atropine was not explored.

The magnitude of the atropine blockade of p.t.p. was dependent on the depth of blockade by hexamethonium. In the presence of 10^{-3}M hexamethonium, the pre-train compound action potential was small, but the p.t.p. was still very conspicuous. Under these circumstances, 10^{-6}M atropine nearly abolished the p.t.p. This may be due to the fact that with deep blockade by hexamethonium, both mechanisms of p.t.p. (muscarinic and non-muscarinic) must occur for p.t.p. to be observed.

Potentiation by McN-A-343

Muscarinic agonists can stimulate autonomic ganglia (Koppanyi, 1932; Ambache, Perry & Robertson, 1956; Takeshige & Volle, 1964). McN-A-343, a muscarinic cholinoceptor agonist, potentiated the compound action potential (Figure 7) in the presence of 5×10^{-4}M hexamethonium. The magnitude of the potentiation was dependent on the depth of blockade in a manner similar to the p.t.p. The duration of the potentiation by McN-A-343 (10^{-4}M for 6 s) was 8 to 12 min. The potentiation was completely blocked by 10^{-6}M atropine.

McN-A-343 also induced discharges in the postganglionic nerve; however, the duration of the discharges was only 1 to 3 min (also see Figure 5, Christ, 1977b). If it is assumed that both the potentiation and the discharges are produced by activation of muscarinic receptors in the ganglion, then the potentiation must be a more sensitive indicator of the actual muscarinic activation.

Post-tetanic potentiation of DMPP discharges

DMPP, a nicotinic cholinoceptor agonist, depolarizes the ganglion cell and induces discharges in the postganglionic nerve of the stellate ganglion (Christ, 1977b). The depolarization by DMPP will be altered by any procedure which alters the postsynaptic membrane. In the presence of hexamethonium, a high concentration of DMPP (3×10^{-4}M) was used to induce discharges (Figure 8). These discharges were potentiated by repetitive stimulation (30 Hz, 5 s). The potentiation of DMPP discharges was completely blocked by atropine (10^{-6}M).

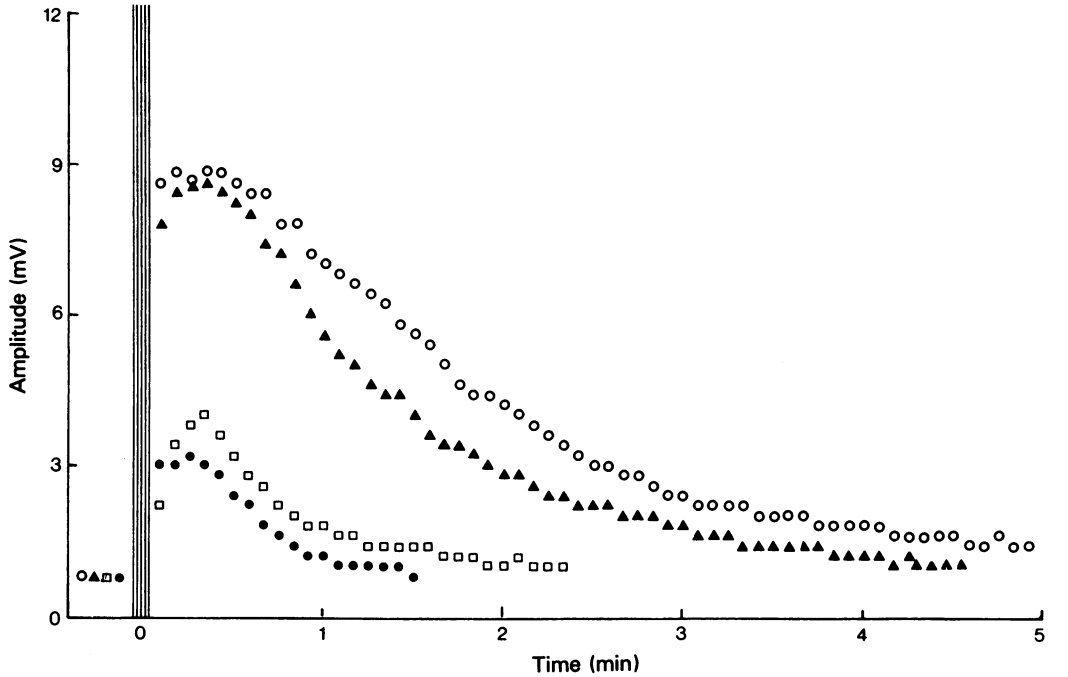

Figure 6 Effect of atropine on the post-tetanic potentiation in hexamethonium (5×10^{-4}M). At the bar, the preganglionic nerve was stimulated at 40 Hz for 5 s in: (○) = control, (▲) = 10^{-8}M atropine, (●) = 10^{-7}M atropine, and (□) = 10^{-6}M atropine. Each point represents the amplitude of one potential and all the results are from a single preparation.

Discussion

The p.t.p. is relatively small in unblocked stellate ganglia. In the unblocked ganglion, transmission may be supraliminal in most synapses, so that potentiation can not be readily observed. Libet (1964) and Brimble & Wallis (1974) observed that submaximal stimulation produces a larger p.t.p. than supramaximal stimulation. This would indicate that a large safety factor of transmission is a limiting factor for p.t.p. in the rabbit superior cervical and cat stellate ganglion. The observation that high concentrations of nicotine and hexamethonium are necessary to block the hamster stellate ganglion also supports the theory that synapses in this ganglion have a large safety factor of transmission.

P.t.p. of ganglionic transmission increases as the depth of blockade by hexamethonium is increased. The depth of blockade does not affect the absolute maximum amplitude of the potentiated evoked potential until very deep levels of blockade are attained (greater than 80%). The depth of blockade does, however, affect the time to reach the maximal potentiation and the rate of recovery from the potentiation. The observation that the rate of recovery is directly related to the depth of blockade indicates that hexa-

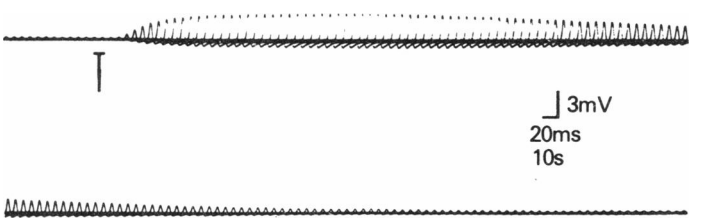

Figure 7 Potentiation of ganglionic transmission by McN-A-343 in the presence of 5×10^{-4}M hexamethonium. At the bar, McN-A-343 was applied at a concentration of 10^{-4} M for 6 s.

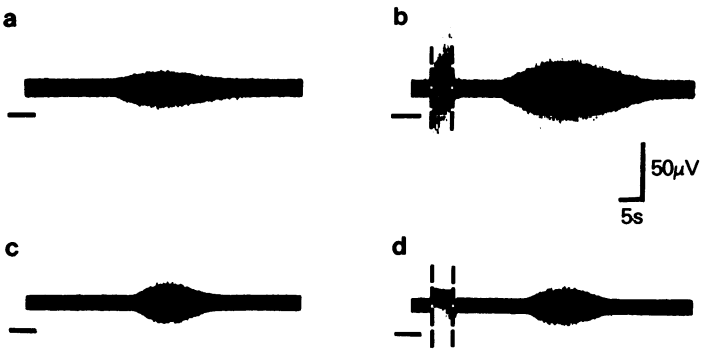

Figure 8 Effect of repetitive preganglionic stimulation (30 Hz for 5 s between the vertical dashed lines) on the postganglionic discharges induced by DMPP (3×10^{-4}M at horizontal bars). The delay in the onset of discharges is primarily due to the dead space in the superfusion chamber. The upper pair of records (a and b) was recorded in the presence of hexamethonium (5×10^{-4}M) in one preparation and the lower pair of records (c and d) was recorded in the presence of hexamethonium (5×10^{-4}M) and atropine (10^{-6}M) from a different preparation. Note the potentiation of the DMPP-discharges in hexamethonium (b), but the absence of potentiation in hexamethonium and atropine (d)

methonium is merely unmasking the p.t.p. In other words, hexamethonium shifts many cells from the discharge zone to the subliminal fringe. Lees & Nishi (1972) have concluded that hexamethonium has no effect on the p.t.p. of the e.p.s.p. in the rabbit superior cervical ganglion. The results in this paper can be explained without hypothesizing that hexamethonium has an effect on the underlying mechanisms of the p.t.p.

The p.t.p. is able to reverse 80% blockade of ganglionic transmission by hexamethonium. This indicates that the mechanisms for p.t.p. occur in at least 80% of the ganglion cells which transmit activity. At 80% blockade, the most sensitive synapses must be quite depressed by hexamethonium, yet the depression is reversed by the p.t.p. Thus, the magnitude of the underlying mechanisms of the p.t.p. are probably very large.

The mechanisms of the p.t.p. in hexamethonium appear to be similar to those described by Libet (1964) for unblocked ganglia. A high-frequency train of stimuli will generate a slow e.p.s.p. in autonomic ganglia. The slow e.p.s.p. can summate with the e.p.s.p. from a single stimulus, thereby raising the e.p.s.p. to a suprathreshold amplitude for the ganglion cell. Alternatively, a reduction in membrane conductance during the slow e.p.s.p. could increase the amplitude of the e.p.s.p. so that it can become suprathreshold (Schulman & Weight, 1976). The involvement of the muscarinic slow e.p.s.p. is supported by several observations. Firstly the p.t.p. is sensitive to atropine; secondly the sensitivity of the ganglion to DMPP is increased after a train of stimuli, as would be expected if the train produces a slow-e.p.s.p. Thirdly, McN-A-343, a muscarinic agonist, produces a potentiation which is similar to the p.t.p.

The fact that the p.t.p. is not completely suppressed by atropine indicates that there is probably an additional mechanism involved in its production. It is possible that a non-cholinergic neurotransmitter is released (Nishi & Koketsu, 1968; Chen, 1971; Alkadhi & McIsaac, 1973; Neild, 1978). If a non-cholinergic neurotransmitter is involved, the neurotransmitter should increase the excitability of the postsynaptic membrane. However, the discharges induced by DMPP in the presence of atropine and hexamethonium are not augmented by repetitive preganglionic stimulation. This indicates that there is no change in the excitability of the postsynaptic neurones. Thus, a presynaptic mechanism is probably producing the atropine-resistant component of the p.t.p. This supports the observation of Libet (1964) that the p.t.p. from heterosynaptic stimulation is completely blocked by atropine. If a non-cholinergic neurotransmitter were producing a component of the p.t.p., it is unlikely that the p.t.p. from heterosynaptic stimulation would be completely blocked by atropine. The presynaptic mechanism of p.t.p. may be similar to the mechanism which results in potentiation of the e.p.s.p. in sympathetic ganglia (Lees & Nishi, 1972; Tashiro, Gallagher & Nishi, 1976).

Repetitive stimulation in the presence of hexamethonium can generate a p.t.p. and also asynchronous afterdischarges. The duration of the afterdischarges is only 1 to 5 s (Christ, 1977b), but the duration of the atropine-sensitive component of the p.t.p. is 3 to 5 min. Both responses appear to be due to the slow e.p.s.p.; however, the afterdischarges will only occur

when the slow e.p.s.p. is suprathreshold in amplitude. The p.t.p. will occur for the complete duration of the slow e.p.s.p.; therefore, the atropine-sensitive component of the p.t.p. is probably a better measure of the time-course of the slow-e.p.s.p. than the afterdischarges.

The same rationale will apply for the response to McN-A-343. The discharges from McN-A-343 (10^{-4}M for 6 s) have a duration of 3 to 5 min, but the potentiation from McN-A-343 has a duration of 8 to 12 min. The potentiation is probably more indicative of the duration of the depolarization from McN-A-343.

The p.t.p. may have several consequences, pharmacologically. Firstly, competitive ganglionic blockade can be surmounted by repetitive stimulation. Thus, an increase in autonomic output from the CNS could theoretically, overcome ganglionic blockade. Secondly, ganglia have different sensitivities to competitive ganglionic blocking drugs. This may be due to differences in the safety factor of transmission; however, it may also be due to differences in the frequency of preganglionic activity or due to differences in the magnitude of the underlying mechanisms for p.t.p.

The author would like to express his thanks to Drs Patricia Shinnick-Gallagher and Joel Gallagher for reviewing this manuscript and to Mrs Bonita Vines and Mrs Ellen Tarkington for their secretarial assistance. The donation of McN-A-343 from McNeil Laboratories, Inc. is also appreciated. This investigation was supported by the National Institutes of Health Research Grant NS 10393 from the National Institute of Neurological and Communicative Disorders and Stroke.

References

ALKADHI, K.A. & MCISAAC, R.J. (1973). Non-nicotinic transmission during ganglionic block with chlorisondamine and nicotine. *Eur. J. Pharmac.*, **24**, 78–85.

AMBACHE, N., PERRY, W.L.M. & ROBERTSON, P.A. (1956). The effect of muscarine on perfused superior cervical ganglia of cats. *Br. J. Pharmac. Chemother.* **11**, 442–448.

BRIMBLE, M.J. & WALLIS, D.I. (1974). The role of muscarinic receptors in synaptic transmission and its modulation in the rabbit superior cervical ganglion. *Eur. J. Pharmac.* **29**, 117–132.

BRIMBLE, M.J., WALLIS, D.I. & WOODWARD, B. (1972). Facilitation and inhibition of cell groups within the superior cervical ganglion of the rabbit. *J. Physiol.*, **226**, 629–652.

BRONK, D.W., TOWER, S.S., SOLANDT, D.Y. & LARRABEE, M.G. (1938). The transmission of trains of impulses through a sympathetic ganglion and in its postganglionic nerves. *Am. J. Physiol.*, **122**, 1–15.

CHEN S. (1971). Transmission in superior cervical ganglion of the dog after cholinergic suppression. *Am. J. Physiol.*, **221**, 209–213.

CHRIST, D. (1977a). Post-tetanic potentiation in the stellate ganglion during blockade with hexamethonium. *Pharmacologist*, **19**, 198.

CHRIST, D. (1977b). Effects of halothane on ganglionic discharges. *J. Pharmac. exp. Ther.*, **200**, 336–342.

ECCLES, R.M. (1952). Action potentials of isolated mammalian sympathetic ganglia. *J. Physiol.*, **117**, 181–195.

KOPPANYI, T. (1932). Studies on the synergism and antagonism of drugs. I. The non-parasympathetic antagonism between atropine and the miotic alkaloids. *J. Pharmac. exp. Ther.*, **46**, 395–405.

LARRABEE, M.G. & BRONK, D.W. (1947). Prolonged facilitation of synaptic excitation in sympathetic ganglia. *J. Neurophysiol.*, **10**, 139–154.

LEES, G.M. & NISHI, S. (1972). Analysis of the mechanism of action of some ganglion-blocking drugs in the rabbit superior cervical ganglion. *Br. J. Pharmac.*, **46**, 78–88.

LIBET, B. (1964). Slow synaptic responses and excitatory changes in sympathetic ganglia. *J. Physiol.*, **174**, 1–25.

LILEY, A.W. (1956). An investigation of spontaneous activity at the neuromuscular junction of the rat. *J. Physiol.*, **132**, 650–666.

NEILD, T.O. (1978). Slowly-developing depolarization of neurones in the guinea-pig inferior mesenteric ganglion following repetitive stimulation of the preganglionic nerves. *Brain Res.*, **140**, 231–239.

NISHI, S. & KOKETSU, K. (1968). Early and late afterdischarges of amphibian sympathetic ganglion cells. *J. Neurophysiol.*, **31**, 109–121.

SCHULMAN, J.A. & WEIGHT, F.F. (1976). Synaptic transmission: Long-lasting potentiation by a postsynaptic mechanism. *Science, N.Y.*, **194**, 1437–1439.

TASHIRO, N., GALLAGHER, J.P. & NISHI, S. (1976). Facilitation and depression of synaptic transmission in amphibian sympathetic ganglia. *Brain Res.*, **118**, 45–62.

TAKESHIGE, C. & VOLLE, R.L. (1964). Modification of ganglionic responses to cholinomimetic drugs following preganglionic stimulation, anticholinesterase agents, and pilocarpine. *J. Pharmac. exp. Ther.*, **146**, 335–343.

(Received July 9, 1979.
Revised August 30, 1979.)