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NEUROPHYSIOLOGY AND PHARMACOLOGY OF LONG-TERM POTENTIATION IN THE RAT SYMPATHETIC GANGLION

BY CLARK A. BRIGGS, THOMAS H. BROWN AND DONALD A. MCAFEE

From the Division of Neurosciences, Beckman Research Institute of the City of Hope, 1450 East Duarte Road, Duarte, CA 91010, U.S.A.

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SUMMARY

1. Brief tetanic stimulation of the preganglionic nerve induced a persistent potentiation of nicotinic synaptic transmission in the rat superior cervical sympathetic ganglion.

2. Quantitative measurements of the post-tetanic increase in synaptic efficacy revealed two distinct time courses. The early, rapidly decaying component, termed post-tetanic potentiation (p.t.p.), had a decay time constant of 2–3 min, as reported elsewhere. The duration of the more persistent component, called long-term potentiation (l.t.p.), was extremely temperature dependent, lasting much longer at 32 °C than at 22 °C. In half of the experiments performed at 32 °C, l.t.p. showed no detectable decay over the course of 1 h or more after a brief tetanic stimulation. Other experiments were conducted at 22 °C.

3. The induction of l.t.p. was dependent on the extracellular [Ca^{2+}]. Transient elevation of the extracellular [K^+] also produced a long-term enhancement of synaptic efficacy, and this effect was also Ca^{2+} dependent.

4. The tetani that were effective in inducing l.t.p. (5–20 Hz for 5–20 s) were well within the physiological range of preganglionic activity. The magnitude and time course were related to frequency and duration of stimulation.

5. The occurrence of l.t.p. was restricted to those preganglionic fibres that were tetanically stimulated. This lack of heterosynaptic or generalized effects was demonstrated by splitting the preganglionic nerve into two branches that could be independently tested and conditioned.

6. Physiological activation of muscarinic or nicotinic receptors apparently does not play an essential role in causing ganglionic l.t.p., which is expressed as an enhancement of nicotinic transmission. A muscarinic antagonist (2 μM-atropine) did not block l.t.p. Preganglionic stimulation induced l.t.p. even when a high concentration of a nicotinic antagonist (3 mM-hexamethonium) was present *during* the tetanic stimulation. Furthermore, bath application of a cholinergic agonist (100–1000 μM-carbachol) could not substitute for tetanic stimulation in provoking l.t.p.

7. Activation of adrenergic receptors also appeared not to play an essential role. Neither a β-adrenergic antagonist (10 μM-sotolol or 1 μM-propranolol) nor an α-adrenergic antagonist (1 μM-phentolamine) had any significant effect on the magnitude or duration of l.t.p.

8. The results indicate that ganglionic l.t.p. is a Ca^{2+}- and temperature-dependent process that can be created independently of the activation of nicotinic, muscarinic or adrenergic receptors.

INTRODUCTION

Long-term potentiation (l.t.p.) is an increased synaptic efficacy that lasts for hours following a few seconds of repetitive stimulation of the presynaptic fibres (Bliss, 1979). This great difference between the duration of usage and the duration of the resultant plasticity is a defining characteristic of l.t.p. and a feature that has led many investigators to suggest that this form of synaptic memory plays an important role in regulating the transmission of information in the nervous system (for reviews see Chung, 1977; Swanson, Teyler & Thompson, 1982; Eccles, 1983; Voronin, 1983; Teyler & Discenna, 1984).

It is now clear that l.t.p. occurs in a variety of central and peripheral nervous tissues (Lewis, Teyler & Shashoua, 1981; Brown & McAfee, 1982; Lee, 1982; Gerren & Weinberger, 1983; Racine, Milgram & Hafner, 1983; Baxter, Bittner & Brown, 1984). Indeed, Dunant & Dolivo (1968; Dunant, 1969) noted that brief tetanic stimulation induced a long-lasting potentiation of nicotinic transmission in the rat superior cervical ganglion several years before the first detailed description of l.t.p. in the hippocampus (Bliss & Lomo, 1973; Bliss & Gardner-Medwin, 1973).

The rat superior cervical ganglion, as originally described by Larrabee & Posternak (1952), is a peripheral sympathetic tissue that offers several advantages for investigating the physiology and pharmacology of synaptic transmission in general (McAfee, 1982), and the mechanisms underlying use-dependent forms of neuroplasticity in particular (Zengel, Magleby, Horn, McAfee & Yarowsky, 1980; Brown & McAfee, 1982). Its well-characterized nicotinic cholinergic synapse lies between distinct input (preganglionic) and output (post-ganglionic) nerves. In this report, we show that ganglionic l.t.p. is a Ca^{2+}- and temperature-dependent process and that the induction of l.t.p. does not apparently depend upon the activation of cholinergic or adrenergic receptors.

METHODS

Superior cervical ganglia were isolated from Sprague–Dawley rats of either sex (170–250 g) and were desheathed and maintained *in vitro* by superfusion (1 ml/min) with oxygenated Locke solution as previously described (McAfee, 1982). Unless noted otherwise, all experiments were conducted at ambient temperature (21–23 °C) and atropine (2 μM) was included in the superfusate to block muscarinic responses (Libet & Tosaka, 1970).

Bipolar suction electrodes were used for stimulating the preganglionic (cervical sympathetic) nerve and for recording compound action potentials from the post-ganglionic (internal carotid) nerve (McAfee, 1982). Preganglionic stimulation was delivered by single monophasic square-wave current pulses, which were 500 μs in duration and supramaximal in intensity unless indicated otherwise. In some experiments, stimulation was made submaximal by reducing the pulse duration. Each preganglionic stimulus elicited a single post-ganglionic compound action potential, providing the measure of ganglionic transmission. These responses were recorded with a bandpass of 0–10 kHz. An on-line, real-time data-acquisition system (Analog Devices) set the interval timing, measured the amplitude and integral of the positive deflexion of the compound action potential, and stored the data for subsequent analysis. The digital data were monitored and compared to the analog signal during each experiment, in order to verify the accuracy of the automated data acquisition.

The integral of the compound action potential, unlike the amplitude, is insensitive to changes

in the synchrony of discharge. Both the amplitude and integral increased during l.t.p. However, the results presented here are based on amplitude measurements because this measurement is most commonly used in other laboratories.

Protocols

In general, the approach to eliciting and measuring l.t.p. was first to test the responsiveness of the ganglion to stimuli at a low frequency, then briefly to tetanize the nerve, and finally to resume testing at the same low frequency. The test frequency was 1/min unless indicated otherwise, and the tetanic stimulation was usually 5 or 20 Hz for 20 s. The duration of the pretetanic control period was 0·5–2 h. This prolonged control period was important to ensure reproducibility of the response and to provide an adequate base line for comparison to the post-tetanic period, which normally lasted for 1 h or more. In some experiments, preganglionic tetanic stimulation was replaced by transient exposure of the ganglion to superfusates containing carbachol, a catecholamine, or high concentrations of K^+.

To detect an enhanced synaptic efficacy, the pretetanic test stimuli must evoke a submaximal post-synaptic response. This is readily accomplished by submaximal stimulation of the presynaptic fibres, as is done in *in vitro* studies of the hippocampus. However, a potential problem with submaximal presynaptic stimulation is that post-tetanic increases in synaptic efficacy could be due to recruitment of the number of presynaptic fibres that are stimulated. In some experiments, we recorded the preganglionic compound action potential from a section of the nerve between the stimulating electrode and the ganglion (cf. Brown & McAfee, 1982). However, when submaximal stimulation was used, the preganglionic signal was frequently too small to measure accurately. In addition, the post-ganglionic response amplitudes were rather variable with this method, possibly due to fluctuating numbers of stimulated preganglionic fibres. Previous studies showed that the preganglionic fibres were not recruited by tetanic stimulation (20 Hz for 20 s) when supramaximal preganglionic stimulation was used (Brown & McAfee, 1982). We therefore used other methods that permitted supramaximal preganglionic stimulation but that nevertheless resulted in submaximal post-synaptic responses before tetanic stimulation. These other methods included addition of a nicotinic cholinergic antagonist (100–150 μM-*d*-tubocurarine or 200–300 μM-hexamethonium), partial transection of the preganglionic nerve, or use of lowered $[Ca^{2+}]$ or elevated $[Mg^{2+}]$. The preferred technique was application of a nicotinic antagonist and supramaximal preganglionic stimulation for both test and tetanic stimuli. The partially transected or split preganglionic nerve afforded the least control over the degree of pretetanic responsiveness. Lowered $[Ca^{2+}]$ or elevated $[Mg^{2+}]$ was less satisfactory because l.t.p. is difficult to induce under these conditions. Regardless of the method used, we attempted to reduce the post-ganglionic response amplitude to approximately 40 % of its maximal value before the tetanic stimulation.

Ganglionic l.t.p. was measured under a variety of experimental conditions. A bracketting technique was generally employed such that each ganglion was conditioned at least three times. The first and third treatments were under identical conditions. During the second treatment, one variable was altered, such as the testing frequency, the conditioning parameters, or a drug. This design was used to control for changes in the physiology of the ganglion over the course of the experiment. However, in fact, we found the first and third treatments yielded comparable results even when separated by an interval of 8 h or more.

Data analysis

The degree of potentiation $I(t)$ was computed as the fractional increase in the post-ganglionic response at time t after the tetanic conditioning stimulation such that

$$I(t) = (V_t - V_c)/V_c, \tag{1}$$

where V_t is the amplitude of the post-ganglionic compound action potential at time t, and V_c is the mean amplitude of at least five control responses obtained just before the tetanic stimulation.

The potentiation of ganglionic transmission subsequent to the tetanic stimulation decayed, at 22 °C, along a time course that was closely described by the sum of two exponential terms,

$$I(t) = P[\exp(-t/\tau_P)] + L[\exp(-t/\tau_L)]. \tag{2}$$

The coefficients P and L were used to quantify the extrapolated magnitudes of post-tetanic

potentiation (p.t.p.) and l.t.p., respectively, immediately following the tetanic stimulation. The time constants τ_P and τ_L provided a quantitative measure of the longevity of p.t.p. and l.t.p., respectively. These parameters were determined by standard regression methods (see Fig. 1).

Solutions

The standard Locke solution contained (in mM): NaCl, 136; KCl, 5·6; $CaCl_2$, 2·2; $MgCl_2$, 1·2; NaH_2PO_4, 1·2; $NaHCO_3$, 20·0; and dextrose, 7·6. Atropine (2 μM) was included routinely. In some experiments, $CaCl_2$ was omitted and $MgCl_2$ was increased to 8 mM. For Locke solution with elevated $[K^+]$, KCl was substituted for NaCl on an equimolar basis to a final concentration of 50 mM-K^+. All solutions were equilibrated with 95% O_2–5% CO_2 and had a pH of 7·2–7·4 at 20–33 °C.

Chemicals and sources

The compounds and their sources were: atropine sulphate, *d*-tubocurarine chloride, hexamethonium bromide, carbachol chloride, (−)-isoprenaline hydrochloride, (−)-noradrenaline hydrochloride and (±)-propranolol hydrochloride (Sigma Chemical Co., St. Louis, MO, U.S.A.); phentolamine mesylate U.S.P. (CIBA Pharmaceutical, Summit, NJ, U.S.A.); and sotalol hydrochloride (gift from Mead-Johnson).

RESULTS

Neurophysiology of l.t.p.

A representative example of ganglionic l.t.p. is illustrated in Fig. 1. Following a control period, during which the post-ganglionic response amplitudes were tested by delivering single preganglionic shocks at 1/min, the preganglionic nerve was tetanically stimulated for 20 s at 20 Hz. After the tetanic stimulation, the post-ganglionic response amplitudes were again tested at 1/min. As indicated, the brief tetanic stimulation enhanced ganglionic transmission for the duration of the experiment.

In this experiment we used supramaximal preganglionic stimulation and the post-ganglionic response was made submaximal by including *d*-tubocurarine (100 μM) in the bathing medium throughout the entire experiment (see Methods for rationale). Because we were interested in l.t.p. of nicotinic synaptic transmission, 2 μM-atropine was also present throughout the experiment in order to block muscarinic responses to preganglionic stimulation (cf. Libet & Tosaka, 1970).

The post-tetanic enhanced transmission decayed relatively rapidly at first and then more slowly for the remainder of the 1 h observation. These two rates of decay are even more apparent when the potentiation is plotted on semilogarithmic coordinates (Fig. 1*B* and *C*). The rapidly decaying component, termed post-tetanic potentiation (p.t.p.), had a decay time constant τ_P of 2·5 min and an extrapolated initial magnitude P of 1·2 (220% of pretetanic control). In contrast, the slowly decaying component, l.t.p., relaxed with a time constant τ_L of 105 min and had an extrapolated initial magnitude L of 0·55 (155% of pretetanic control). As shown by the continuous curve in Fig. 1*C*, the time course of the post-tetanic change was accurately described by the sum of two exponentials (eqn. 2).

Although in this experiment we used a nicotinic antagonist to produce a submaximal post-ganglionic response, the occurrence of ganglionic l.t.p. is not critically dependent upon the particular method used to produce a submaximal post-ganglionic response (Table 1 and Figs. 2–5). We have observed ganglionic l.t.p. in twenty-one of twenty-five experiments using submaximal preganglionic stimulation; in eleven of eleven experiments using a partially transected or split preganglionic nerve; and in 180 of 180 experiments with the addition of a nicotinic antagonist. Normally, we preferred

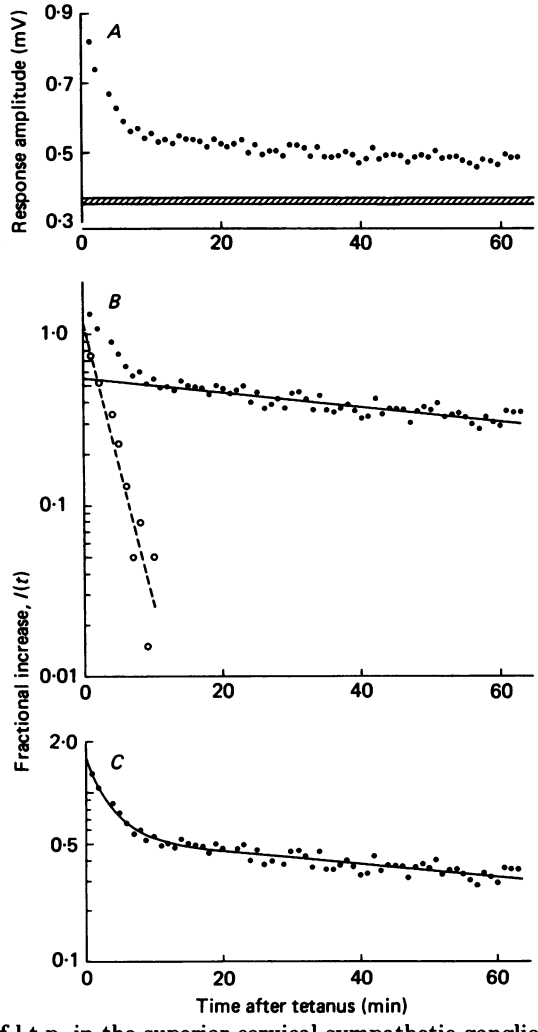

Fig. 1. Analysis of l.t.p. in the superior cervical sympathetic ganglion (22 °C). Atropine (2 μM) and *d*-tubocurarine (100 μM) were present throughout and stimulation was supramaximal. The preganglionic nerve was tetanically stimulated at 20 Hz for 20 s (at 0 min) and transmission was tested before and after the tetanus with one preganglionic stimulus every 60 s. *A*, the amplitude of each post-ganglionic compound action potential is plotted as a function of time after tetanic preganglionic stimulation (●). Also shown is the mean ± S.D. of five responses obtained immediately before the tetanic stimulation (control, hatched bar). *B*, the fractional increase in the post-ganglionic compound action potential amplitude after tetanic stimulation is shown (●; eqn. (1), Methods). Also shown is the fractional increase after subtraction of the slowly decaying component (○). These data were analysed using eqn. (2) and application of standard regression analysis. The following parameters were extracted: $P = 1{\cdot}2$, $\tau_P = 2{\cdot}5$ min, $L = 0{\cdot}55$ and $\tau_L = 105$ min. *C*, the total fractional increase is replotted and the continuous line is a theoretical curve computed using eqn. (2) and the above estimates of P, τ_P, L and τ_L. As indicated, the double-exponential function (eqn. 2) provides an excellent fit to the experimental data.

not to use submaximal preganglionic stimulation. This technique makes it difficult to assess the possible role of preganglionic fibre recruitment in the observed post-tetanic effect.

Temperature dependence of l.t.p. In experiments performed at 22 °C, ganglionic l.t.p. took at least 1 h to decay to 5% of initial value (3 times τ_L; see Table 1). However, given enough time, the post-ganglionic response amplitude always decayed back to the control value. In contrast, l.t.p. in the hippocampal slice sometimes shows little or no detectable decay over the course of 1 h (see Barrionuevo & Brown, 1983). An important difference is that studies in the hippocampus are carried out at 32–37 °C. When we raised the temperature of the superfusate bathing the ganglion, the persistence of ganglionic l.t.p. increased dramatically.

In the experiment illustrated in Fig. 2, we reduced ganglionic transmission by partial transection of the preganglionic nerve. Following a control period, l.t.p. was induced by supramaximal stimulation at 20 Hz for 20 s. When the experiment was performed at 23 °C, l.t.p. decayed with a double-exponential time course. The decay time constant for the l.t.p. component, τ_L, was 81 min. After l.t.p. had decayed back to the control level, the experiment was repeated in the same ganglion at 31 °C. At this temperature there was no decay of the potentiation. In fact, there was actually a gradual increase in transmission.

Regardless of the method used to produce submaximal post-ganglionic responses, the duration of l.t.p. was much longer in experiments performed at 32 °C than at 22 °C. In the sixteen experiments summarized in Table 1, at 22 °C l.t.p. always decayed and the mean value of τ_L was 35 min. At 32 °C, there was no detectable decay (denoted n.d. in Table 1) of l.t.p. in eight of the sixteen experiments (see Fig. 3). In the eight remaining experiments in which there was some detectable decay of l.t.p. at 32 °C, the average value of τ_L was 14 h.

These effects were clearly due to temperature and not the consequence of tetanically stimulating the same ganglion twice. When l.t.p. was induced twice in the same ganglion at 22 °C, the duration of l.t.p. normally was not longer after a second tetanic stimulation.

The prolonged nature of l.t.p. at 32 °C presents a problem for certain types of experimental investigations. When the potentiation fails to decay back to the control level at a reasonable rate, a second treatment cannot be conveniently performed in the same ganglion if one desires a similar pre-potentiation level of transmission. Therefore, the subsequent experiments were conducted at ambient temperature (21–23 °C).

Ca^{2+} dependence of l.t.p. The induction of ganglionic l.t.p. is a Ca^{2+}-dependent process. This is clearly demonstrated in Fig. 4. When Ca^{2+} influx was prevented during the tetanus by stimulating in a medium containing no added Ca^{2+} and 8 mM-Mg^{2+}, l.t.p. was not observed upon return to normal medium. However, retetanization of the same ganglion in normal medium did induce l.t.p. In addition to brief preganglionic tetanic stimulation, we found that transient exposure of the ganglion to an elevated concentration of extracellular K^+ induced a long-term increase in synaptic efficacy. This K^+-induced effect was similarly Ca^{2+} dependent (Fig. 4).

Dependence of l.t.p. on parameters of tetanic stimulation. L.t.p. can be induced by a variety of stimulus frequencies and durations. We compared stimulus trains of 5 Hz

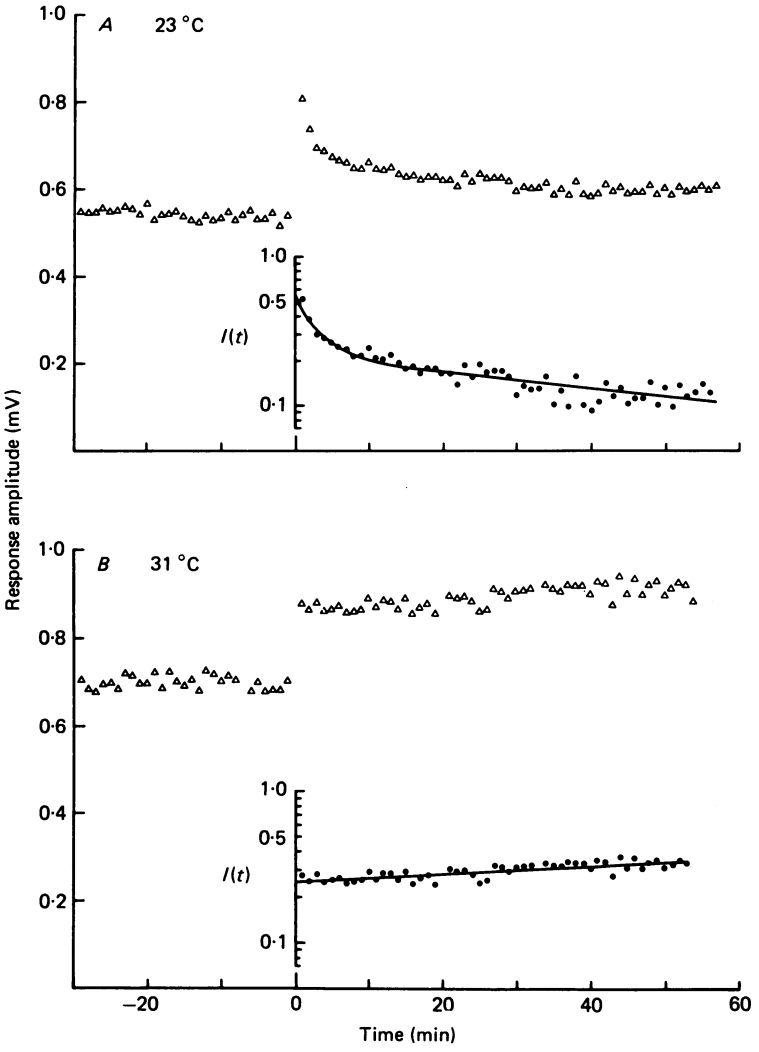

Fig. 2. Effect of temperature on l.t.p. Preganglionic stimulation was supraximal and transmission was reduced by partial transection of the preganglionic nerve. All data are from the same ganglion, at both 23 (*A*) and 31 °C (*B*). At each temperature, the preganglionic nerve was tetanically stimulated at 20 Hz for 20 s (at 0 min). Shown are the amplitudes of the post-ganglionic compound action potential responses before and after the tetani, and, in insets, the fractional increase in transmission ($I(t)$) after tetanic stimulation. At 23 °C the post-tetanic enhancement of transmission decayed as a double exponential with the following parameters: $P = 0{\cdot}32$, $\tau_P = 3{\cdot}2$ min, $L = 0{\cdot}21$ and $\tau_L = 81$ min.

for 20 s (100 pulses), 20 Hz for 5 s (100 pulses), and 20 Hz for 20 s (400 pulses) in a paired fashion. As shown in Table 2, stimulation with 100 pulses at 20 Hz produced l.t.p. that was slightly greater in magnitude than that produced by the same number of pulses at a lower frequency (5 Hz). The magnitude of l.t.p. produced by stimulation with 400 pulses at 20 Hz was twofold greater than that produced by fewer stimuli

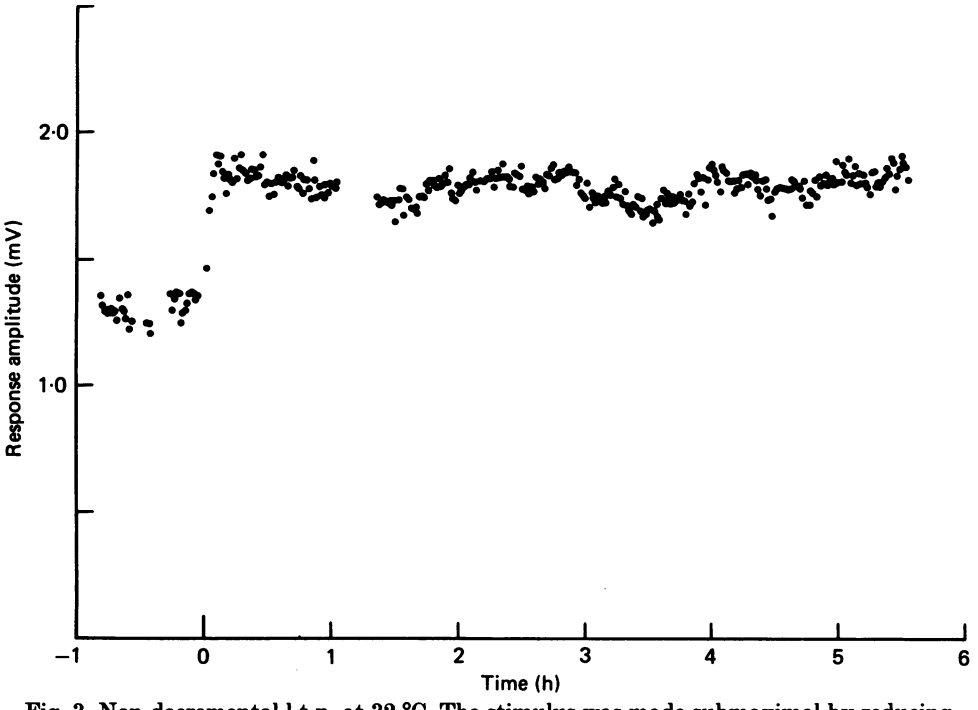

Fig. 3. Non-decremental l.t.p. at 32 °C. The stimulus was made submaximal by reducing the pulse duration from 500 μs (supramaximal) to 38 μs. Tetanic stimulation (20 Hz for 20 s at 0 min) induced a potentiation of the response to submaximal stimulation that lasted for more than 5 h, as shown. The response to supramaximal stimulation did not change. This was measured before the tetanic stimulation and between 63 and 80 min afterwards.

TABLE 1. Effect of temperature on l.t.p. under three experimental conditions

	Coefficient, L		Time constant, τ_L (min)	
Temperature (°C)	Range	Mean ± S.E. of mean	Range	Mean ± S.E. of mean
Submaximal nerve stimulation (n = 6) 20 Hz, 20 s				
22	0·24–0·66	0·39 ± 0·06	19–36	29 ± 3
32	0·12–0·36	0·22 ± 0·04	162–n.d.†	*
Partially transected nerve (n = 4) 20 Hz, 20 s				
22	0·13–0·27	0·21 ± 0·03	38–81	52 ± 10
32	0·07–0·25	0·15 ± 0·05	71–n.d.†	*
Nicotinic antagonist (n = 6) 5 Hz, 20 s				
22	0·47–1·25	0·66 ± 0·12	28–41	30 ± 3
32	0·21–0·54	0·33 ± 0·06	66–n.d.†	*

Before inducing l.t.p., ganglionic transmission was made submaximal by three different methods: submaximal preganglionic stimulation; partial transection of the preganglionic nerve with supramaximal stimulation; or inclusion of a nicotinic antagonist (250 μM-hexamethonium or 100 μM-*d*-tubocurarine) with supramaximal preganglionic stimulation. Atropine (2 μM) was used to block muscarinic responses. In each preparation, l.t.p. was induced at both 22 and 32 °C by tetanic stimulation at 20 or 5 Hz for 20 s as indicated.

* L.t.p. at 32 °C did not decay in eight of these sixteen experiments. In the other eight experiments, τ_L was 162 min (submaximal stimulation, n = 1), 99 min (partially transected nerve, n = 3), and 420 min (nicotinic antagonist, n = 4).

† N.d., no decay.

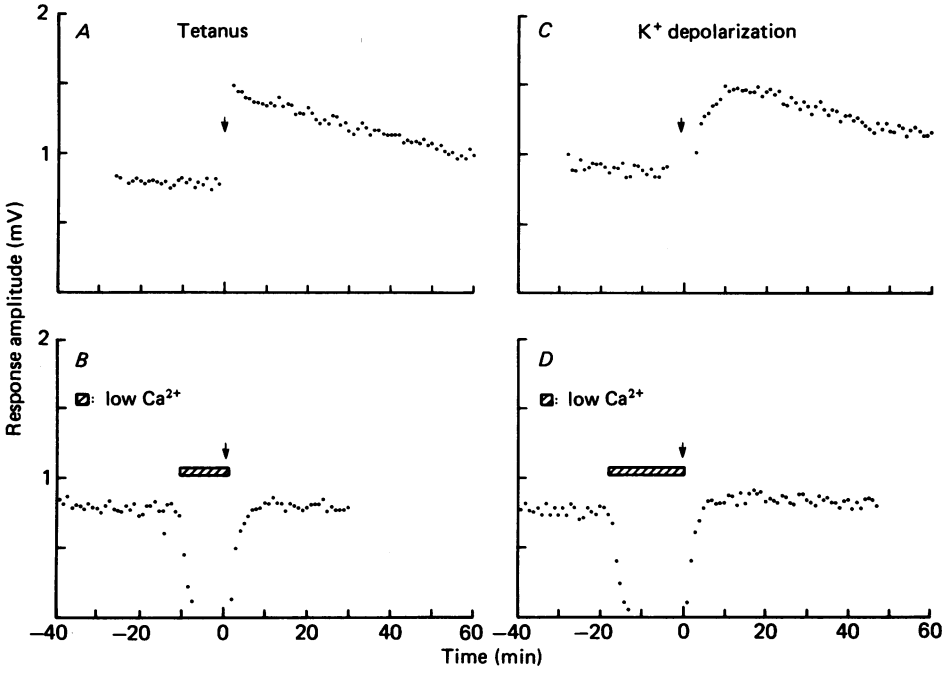

Fig. 4. Ca^{2+} dependence of l.t.p. and the long-lasting enhancement induced by high $[K^+]$. All data are from the same ganglion. Preganglionic stimulation was supramaximal and *d*-tubocurarine (100 μM) was present throughout. *A*, at the arrow, the preganglionic nerve was tetanically stimulated (20 Hz for 20 s). This treatment induced p.t.p. and l.t.p. *B*, test responses were blocked when the ganglion was superfused with a modified Locke medium containing no added Ca^{2+} and 8 mM-Mg^{2+} (hatched bar). The induction of l.t.p. was also blocked when tetanic preganglionic stimulation (20 Hz for 20 s; arrow) was applied in this medium. *C*, instead of tetanic stimulation, the ganglion was briefly depolarized with high K^+ by switching for 3 min to a superfusate of modified Locke solution containing 50 mM-K^+ (arrow). This treatment also induced a long-lasting enhancement of transmission. *D*, this potentiation was blocked when the K^+ pulse (arrow) was applied during superfusion with Locke solution containing no added Ca^{2+} and 8 mM-Mg^{2+} (hatched bar). Transient treatment with the low-Ca^{2+}, high-Mg^{2+} Locke solution itself produced no long-lasting after-effect (not shown). Similar results were obtained in three other experiments.

TABLE 2. Dependence of l.t.p. on parameters of the conditioning tetanus

	Coefficient, L		Time constant, τ_L (min)	
Tetani	Range	Paired difference, mean ± S.E. of mean	Range	Paired difference, mean ± S.E. of mean
20 Hz, 5 s *versus* 5 Hz, 20 s	0·41–0·73 / 0·22–0·52	0·10 ± 0·04*	32–73 / 21–59	9 ± 5
20 Hz, 20 s *versus* 5 Hz, 20 s	0·41–0·77 / 0·16–0·46	0·34 ± 0·05**	33–71 / 25–59	13 ± 4**

The preganglionic nerve was stimulated supramaximally in the presence of 200 μM-hexamethonium. Tetani of 5 Hz for 20 s (100 pulses), 20 Hz for 5 s (100 pulses) and 20 Hz for 20 s (400 pulses) were used to induce l.t.p. Statistical significance was assessed by the two-tailed paired *t* test.

* $P = 0.05$ ($n = 7$). ** $P < 0.02$ ($n = 7$).

at the same frequency. The effects of these stimulus parameters on the time constants were less pronounced.

It is possible to induce l.t.p. consistently with trains as short as twenty-five pulses at 5 Hz ($n = 7$). Clearly, ganglionic l.t.p. can be induced *in vitro* by conditioning parameters well within the physiological range reported *in vivo* (Iggo & Vogt, 1960; Skok, 1980).

Dependence of l.t.p. on testing frequency. In another series of experiments we examined the effect on l.t.p. of the testing frequency. We initially adopted a testing or measuring frequency of 1 stimulus/min because it was sufficiently rapid to follow the time course of l.t.p. and yet seemed infrequent enough to be unlikely to alter the phenomenon. This testing frequency is considerably lower than is commonly used in studies of l.t.p. in other *in vitro* preparations. In each of four ganglia, we found that the decay time constant for l.t.p. (induced by tetanic stimulation at 20 Hz for 20 s) was 3- to 30-fold longer when the testing was done with 1 stimulus/5 s as compared with 1/60 s. Therefore, at least in the ganglion, it appears that the testing frequency is an important parameter in considering the properties of l.t.p.

Restriction of l.t.p. to tetanized synapses. In four experiments, the preganglionic nerve was divided into two branches, and each branch was then pulled into a separate suction electrode for stimulation. The purpose was to determine if tetanic stimulation of one branch causes heterosynaptic l.t.p. in the non-tetanized branch, or whether the induction of l.t.p. is specific to the tetanically stimulated set of preganglionic fibres. It is estimated from studies of mammalian superior cervical ganglia that each preganglionic fibre makes synaptic contact with more than ten post-ganglionic neurones and that the latter are each innervated by synapses from ten or more preganglionic fibres (Gabella, 1976; Njå & Purves, 1977). If l.t.p. were due to generalized post-synaptic changes or to the release of a diffusable factor, one might expect heterosynaptic interactions. However, we found that induction of l.t.p. in either branch alone failed to potentiate responses to stimulation of the other branch (Fig. 5).

Role of cholinergic and adrenergic receptors in l.t.p.

Muscarinic receptors. A long-lasting enhancement of muscarinic responses is known to occur in sympathetic ganglia following repetitive preganglionic stimulation and exposure to certain pharmacological agents (Volle, 1966; Libet, Kobayashi & Tanaka, 1975). If this muscarinic process were requisite in ganglionic l.t.p., one would expect application of 2 μM-atropine to block the phenomenon. The results presented in Table 3 clearly indicate that atropine has little effect on l.t.p.

Nicotinic receptors. Ganglionic l.t.p. is *expressed* as a potentiation of nicotinic transmission *after* the conditioning tetanus. We were interested in knowing whether activating nicotinic receptors *during* the conditioning tetanus is important for the *induction* of l.t.p. This issue was addressed in two ways. One approach was to block the nicotinic receptors during the preganglionic tetanic stimulation by transiently introducing a high concentration of hexamethonium (3 mM). The paradigm was similar to that used to investigate the Ca^{2+} dependence of l.t.p. (Fig. 4). Even though 3 mM-hexamethonium blocked all post-ganglionic evidence of activity, l.t.p. was still induced (Fig. 6). Indeed, the magnitude and duration of this l.t.p. was comparable

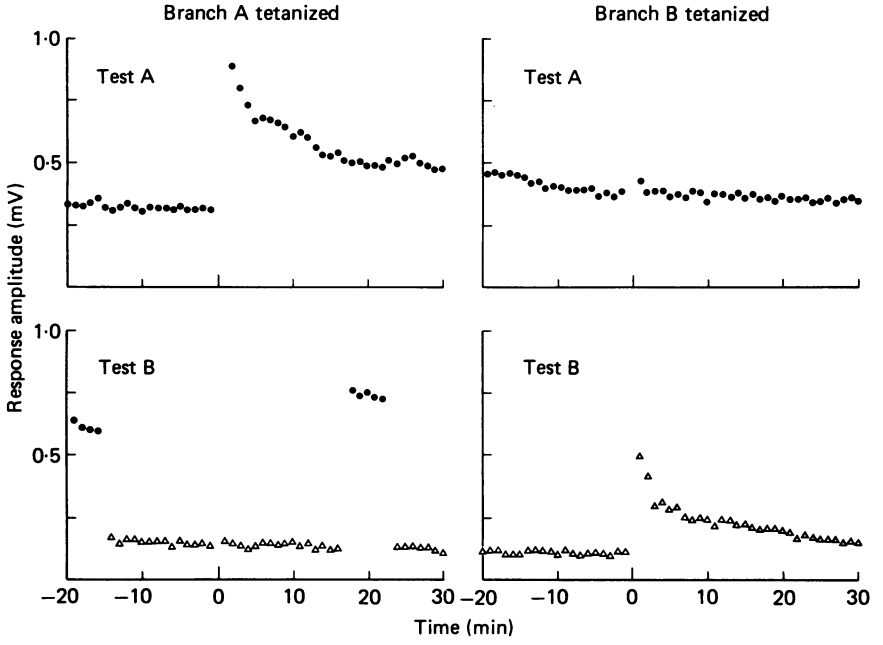

Fig. 5. Lack of a heterosynaptic effect. The preganglionic nerve was split into two branches, A and B, by first sliding the epineurium partway along the nerve towards the ganglion and then teasing apart the exposed nerve fibres with blunt dissection. Each branch was stimulated supramaximally and, in this experiment, hexamethonium (100 μM) was included in the superfusate. Tetanic stimulation (20 Hz for 20 s at 0 min) of branch A potentiated responses to single test stimuli in branch A (upper left panel). Another tetanic stimulation of branch A (lower left panel) further potentiated responses in branch A (●; compare data taken between 18 and 22 min to that taken between −16 and −19 min), but did not potentiate responses to test stimuli in branch B (△). Similarly, tetanic stimulation of branch B potentiated responses to test stimuli in branch B (lower right panel), but another tetanus of branch B did not potentiate the responses to test stimuli in branch A (upper right panel). While l.t.p. could be elicited repeatedly in the tetanized branch, heterosynaptic l.t.p. could not be elicited by the first or the second period of tetanic stimulation. Similar results were obtained in three other experiments. In two experiments no nicotinic antagonist was employed.

TABLE 3. Pharmacology of l.t.p.

	Coefficient, L		Time constant, τ_L (min)	
	Range	Mean ± S.E. of mean	Range	Mean ± S.E. of mean
No atropine	0·44–1·05	0·88 ± 0·12	35–56	43 ± 4
2 μM-atropine	0·33–1·24	0·78 ± 0·15	33–72	52 ± 7
No propranolol	0·38–0·72	0·64 ± 0·07	20–59	36 ± 6
1 μM-propranolol	0·51–0·83	0·61 ± 0·06	27–78	39 ± 10

Transmission was measured with supramaximal stimulation in the presence of *d*-tubocurarine or hexamethonium. L.t.p. was induced by tetani of 20 Hz for 20 s in the presence and absence of atropine in five experiments, and by tetani of 5 Hz for 20 s in the presence and absence of propranolol in another five experiments. Neither antagonist had a statistically significant effect on either L or τ_L (paired t test).

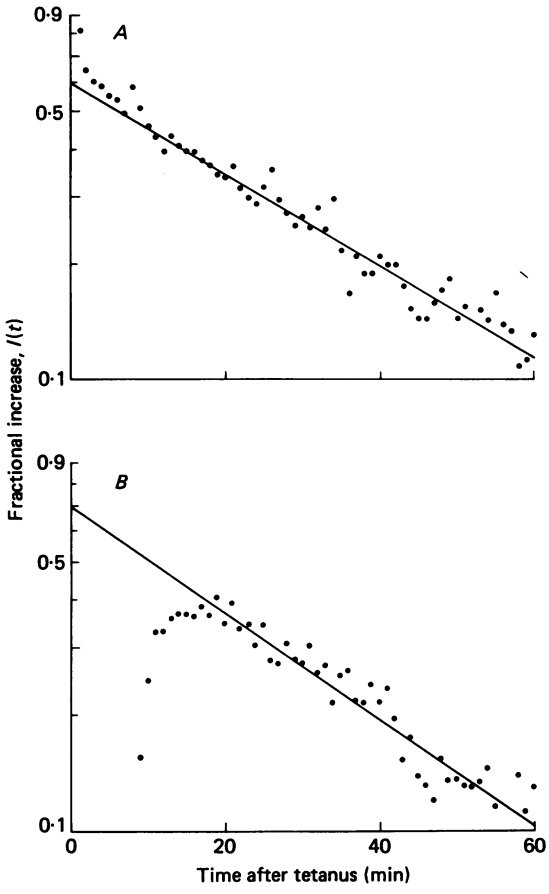

Fig. 6. Blockade of nicotinic receptors during tetani does not reduce l.t.p. The preganglionic nerve was stimulated supramaximally and hexamethonium (200 μM) was present. L.t.p. was induced by preganglionic tetanic stimulation at 5 Hz for 5 s (at 0 min). The fractional increase of the response to supramaximal preganglionic stimulation is plotted as a function of time after each tetanic stimulation. *A*, l.t.p. induced in control Locke solution (200 μM-hexamethonium) had the following parameters: $L = 0{\cdot}59$, $\tau_L = 36$ min. *B*, after increasing the concentration of hexamethonium in the medium to 3 mM, there was no evidence of a post-ganglionic response to test stimuli. The ganglion was then tetanized and the superfusate was returned to control Locke solution containing 200 μM-hexamethonium. Within a few minutes it was clear that the responses to test stimuli had been potentiated in comparison to responses obtained immediately before increasing the hexamethonium. This l.t.p. had the following parameters: $L = 0{\cdot}71$ and $\tau_L = 31$ min. This is quantitatively similar to the l.t.p. induced by tetanic stimulation in control medium. Similar transient blockade of test responses by superfusion of 3 mM-hexamethonium produced no after-effect on transmission in the absence of tetanic stimulation; 15–18 min were required to wash out this high concentration of hexamethonium. Atropine (2 μM) was present during all phases of the experiment. Similar results were obtained in three other experiments (tetani of 5 Hz for 5 or 20 s).

to that induced in the absence of increased hexamethonium in the same ganglion ($n = 4$). Another approach was to stimulate nicotinic receptors directly by transient application of a cholinergic agonist (carbachol, 100–1000 μM, $n = 7$). As shown in Fig. 7, the response to single preganglionic stimuli was composed of a brief spike that was often followed by a slow depolarization. L.t.p. was measured as an increase in

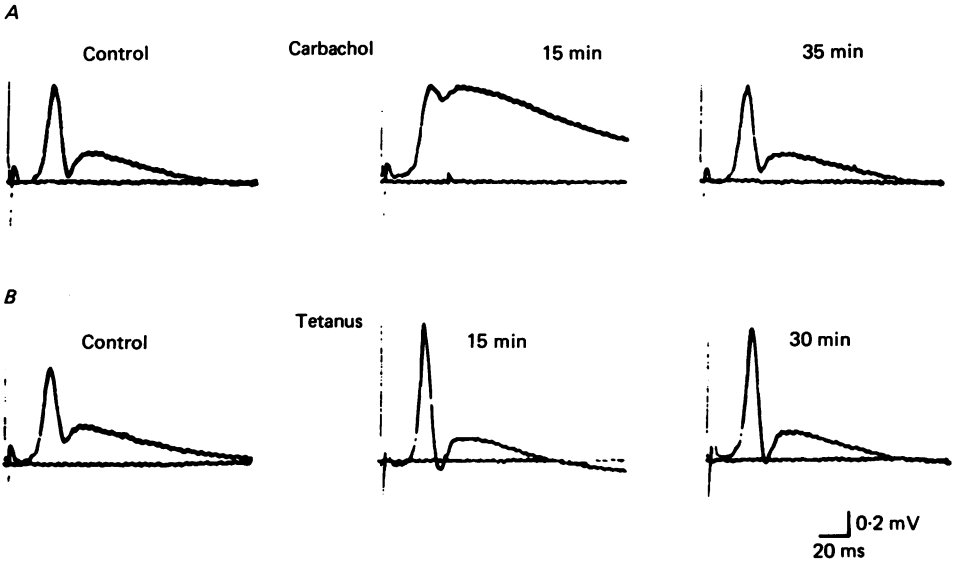

Fig. 7. An exogenous cholinergic agonist does not induce l.t.p. The preganglionic nerve was split and one of the branches was stimulated supramaximally. The response was 42 % of that obtained by stimulating both branches simultaneously. Under our recording conditions, the post-ganglionic compound action potential is a rapid spike exceeding 0·5 mV and lasting about 20 ms. A smaller and slower depolarizing wave can be seen following the compound action potential. The horizontal line in each panel is a base-line trace (0 mV). *A*, shown is a control response obtained immediately before superfusion of 1 mM-carbachol for 3 min, and two responses obtained 15 and 35 min after the superfusion of carbachol. This brief exposure to carbachol did not induce a potentiation of the compound action potential. However, the slow wave following the compound action potential was potentiated at 15 min after tetanus. *B*, subsequent preganglionic tetanic stimulation (20 Hz for 20 s) did induce l.t.p. of the post-ganglionic compound action potential in the same ganglion. Responses obtained at 15 and 30 min after the tetanic stimulation were potentiated by 55 and 43 %, respectively. Atropine (2 μM) was present during all phases of the experiment. Similar results were obtained in six other experiments with 100 μM to 1 mM-carbachol.

the amplitude and integral of the spike. A brief exposure to carbachol potentiated the slow depolarization for up to 20 min, but did not potentiate the spike (cf. Brown, Brownstein & Scholfield, 1972). In contrast, subsequent tetanic preganglionic stimulation did induce long-term potentiation of the spike. Thus, activation of nicotinic receptors appears to be neither necessary nor sufficient to induce ganglionic l.t.p.

Catecholamine receptors. In the mammalian superior cervical ganglion, β-adrenergic agonists cause a depolarization and facilitate synaptic transmission (Brown & Dunn, 1983), but a long-lasting after-effect has not been reported. We found that propranolol

(1 μM), a β-adrenergic antagonist, had no effect on the l.t.p. induced by tetanic preganglionic stimulation (Fig. 8 and Table 3). In addition neither sotalol (10 μM, $n = 3$) another β-adrenergic antagonist, nor phentolamine (1 μM, $n = 6$), an α-adrenergic antagonist, had any effect on l.t.p. Thus, endogenous noradrenaline does not appear to play a role in the l.t.p. induced by preganglionic conditioning at 22 °C.

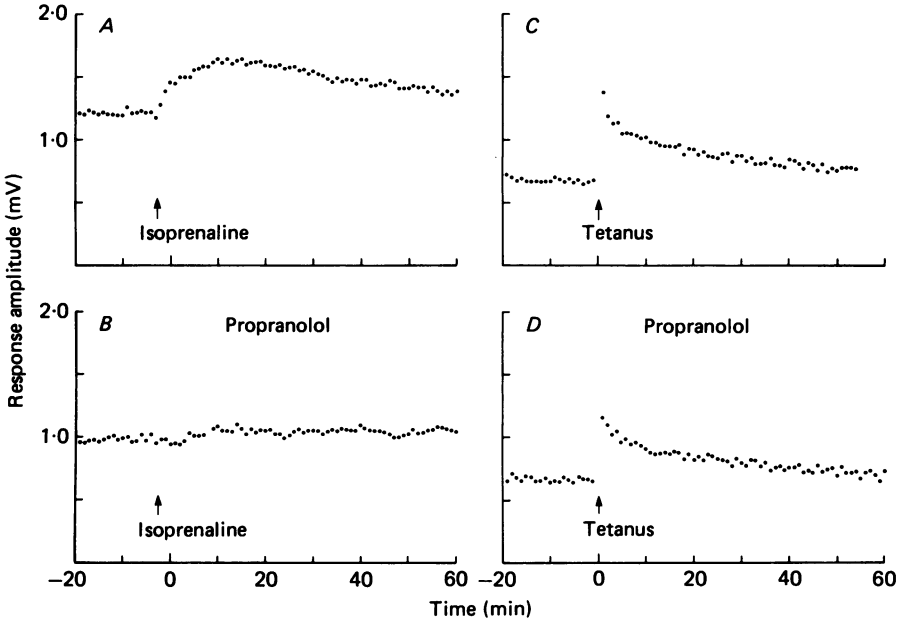

Fig. 8. Blockade of β-adrenergic receptors during tetani does not reduce l.t.p. Preganglionic stimulation was supramaximal. The data in *A* and *B* are from one ganglion (200 μM-hexamethonium present) while the data in *C* and *D* are from another ganglion (300 μM-hexamethonium present). *A*, superfusion was switched for 3 min to Locke solution containing isoprenaline (3 μM; arrow), in addition to hexamethonium and atropine. Transmission was potentiated for at least 1 h. The effect was reproduced in three other experiments. *B*, when propranolol (1 μM) was present throughout, a 3 min pulse of Locke solution containing 3 μM-isoprenaline (arrow; as well as propranolol, hexamethonium, and atropine) was ineffective. This was the largest effect of such a pulse of isoprenaline in the presence of 1 μM-propranolol. In three other experiments, propranolol completely blocked the effect of isoprenaline. *C*, both p.t.p. and l.t.p. were induced by a preganglionic stimulus train at 5 Hz for 20 s (arrow). *D*, propranolol (1 μM) had no significant effect on l.t.p. induced by a second, identical stimulus train. Similar results were obtained in four other experiments.

In other experiments, we superfused the ganglion with isoprenaline (3 μM) for 3 min in place of tetanic stimulation. This treatment did produce a potentiation of transmission that lasted for 1 h or more, and the potentiation was blocked by 1 μM-propranolol (Fig. 8). Such an effect is not necessarily due to the induction of a l.t.p.-like process. Concentrations of isoprenaline as low as 1 nM can produce a measurable potentiation of ganglionic transmission (Brown & Dunn, 1983). Based on our measurements of [^{14}C]mannitol clearance from the ganglion, we find that the observed effect of isoprenaline could simply be due to the time required to reduce

the concentration of isoprenaline in the ganglion to 1 nM. This, together with the failure of propranolol to alter tetanically induced l.t.p., suggests that applied agonists can produce a long-lasting potentiation that may reflect the time required for superfusion to reduce the agonist concentration to below its effective level.

DISCUSSION

While a number of investigators have studied post-tetanic potentiation in autonomic ganglia, few have focused on longer-lasting processes. Libet and colleagues (Libet & Tosaka, 1970; Libet *et al.* 1975; Ashe & Libet, 1981) noted a long-term enhancement of muscarinic responses. However, our experiments were done in the presence of the muscarinic antagonist atropine, and thus represent a long-term enhancement of nicotinic, not muscarinic transmission. Kumamoto & Kuba (1983*a*) have reported an enhancement of acetylcholine sensitivity that can be induced by *post*-ganglionic stimulation of frog lumbar ganglia. This appears to be unlike the process we report here, because non-synaptic stimulation was found not to induce l.t.p. in the rat ganglion (Brown & McAfee, 1982; Briggs, Brown & McAfee, 1983).

Duration and magnitude of l.t.p.

Temperature had a strong influence on the duration of l.t.p., the rate of decay of the potentiation being slower at 32 than at 22 °C. Indeed, the time constant at 32 °C was immeasurably large (longer than 100 h) in approximately half of the experiments. Because of this, we chose to perform most of our experiments at 22 °C so that multiple episodes of l.t.p. could be induced, allowing repeated measures in the same ganglion. In contrast to l.t.p., other post-tetanic processes such as facilitation, augmentation, and post-tetanic potentiation decay more rapidly at higher temperatures (Zengel *et al.* 1980).

A simple interpretation of the temperature dependence of l.t.p. is that the phenomena at 22 and at 32 °C are due to one and the same mechanism, and that this mechanism is longer-lasting at 32 °C. However, it is alternatively possible that more than one mechanism underlies l.t.p. One process may last for hours at both temperatures, while a second process that can potentiate transmission for even longer periods may be apparent only at warmer temperatures. Obviously, further mechanistic studies of l.t.p., especially at 32 °C, are required.

It is clear that l.t.p. in the ganglion can result from very mild conditioning stimulation. We found that tetani with as few as twenty-five stimuli at 5 Hz would reliably induce l.t.p. Increasing the frequency and duration increased the magnitude and to a lesser extent the duration of l.t.p.

Mechanism of l.t.p.

Role of extracellular Ca^{2+}. In the hippocampus, it has been proposed that a post-synaptic influx of Ca^{2+} leads to a long-term increase in neurotransmitter receptors and, thereby, increased synaptic efficacy (Lynch, Halpain & Baudry, 1982; Lynch, Larson, Kelso, Barrionuevo & Schottler, 1983). Recent observations in the frog sympathetic ganglion are consistent with this scheme (Kumamoto & Kuba, 1983*a*). We also find that the induction of ganglionic l.t.p. is dependent upon the

presence of extracellular Ca^{2+}. However, antidromic stimulation induced little or no l.t.p. in the rat ganglion (Brown & McAfee, 1982), and non-synaptic stimulation (intracellular depolarization) of individual post-ganglionic neurones did not potentiate synaptic transmission (Briggs *et al.* 1983). Furthermore, the experiments presented in this paper demonstrate that activation of cholinergic receptors was not necessary for the induction of ganglionic l.t.p., even though activation of these receptors would depolarize post-ganglionic neurones (Kuba & Koketsu, 1978) and thereby increase Ca^{2+} influx through voltage-dependent channels (McAfee & Yarowsky, 1979). Instead, it may be that *presynaptic* rather than *post-synaptic* influx of Ca^{2+} is important for induction of l.t.p. Such a process either could support the release of some substance that is responsible for inducing l.t.p. or could directly trigger a metabolic action within the cholinergic nerve terminals that produces a long-term potentiation of acetylcholine release.

Role of neuromodulators. Recent studies of the hippocampus have suggested that noradrenaline may participate in l.t.p. (Bliss, Goddard & Riives, 1983; Neuman & Harley, 1983; Voronin, 1983; Hopkins & Johnston, 1984) but such results may not be in complete agreement with those of others (Dunwiddie, Roberson & Worth, 1982). Additionally, prolonged exposure of the frog sympathetic ganglion to a high concentration of adrenaline has been reported to induce a long-lasting potentiation of nicotinic transmission (Kuba, Kato, Kumamoto, Koketsu & Hirai, 1981; Kumamoto & Kuba, 1983*b*).

While acetylcholine and noradrenaline are abundant in the sympathetic ganglion, our pharmacological studies indicate that neither substance is required for *inducing* l.t.p. (which is *expressed* as an enhancement of nicotinic synaptic efficacy). L.t.p. was not blocked by the combination of atropine (2 μM), present during the entire experiment, and a high concentration of hexamethonium (3 mM), present during the tetanic stimulation. Carbachol was unable to mimic tetanic stimulation in producing l.t.p. Adrenergic antagonists selective for α- and for β-receptors also did not reduce l.t.p. Furthermore, other investigators have found that dopamine enhances muscarinic but not nicotinic transmission in the superior cervical ganglion (Libet & Tosaka, 1970; Libet *et al.* 1975; Ashe & Libet, 1981).

In a more general approach, we divided the preganglionic nerve into two branches in order to determine whether tetanic stimulation of one set of synapses could induce l.t.p. in another set of synapses. If such heterosynaptic l.t.p. occurred, this would be consistent with the idea of a releasable substance that mediates l.t.p. However, no heterosynaptic l.t.p. could be detected. Thus, ganglionic l.t.p. appears to be either independent of the action of an extracellular neuromodulator, or possibly dependent on a neuromodulator that cannot diffuse far within the ganglion.

Locus of l.t.p. A simple scheme consistent with all of our data is that tetanic stimulation increases $[Ca^{2+}]$ in the cholinergic nerve terminals and this increase in intraterminal Ca^{2+} then elicits a presynaptic modulation responsible for l.t.p. Additionally, other studies indicate that l.t.p. is, at least in part, due to a potentiation of transmitter release. In the hippocampus, tetanic stimulation has been found to cause, in company with l.t.p., a long-lasting increase in the release of tracers for the putative neurotransmitter, [^{3}H]aspartate (Skrede & Malthe-Sorenssen, 1981) and [^{3}H]glutamate (Dolphin, Errington & Bliss, 1982). In the crayfish neuromuscular

junction, l.t.p. is accompanied by an increase in quantal content but not quantal size (Baxter *et al.* 1984). Finally, in the rat superior cervical ganglion, there is some evidence to suggest that l.t.p. is accompanied by an increase in the evoked release of endogenous acetylcholine (McCaman, Briggs & McAfee, 1984).

In the hippocampus, l.t.p. is regulated by co-operative or associative interactions (McNaughton, Douglas & Goddard, 1978; Levy & Steward, 1979; Barrionuevo & Brown, 1983). The processes responsible for these interactions are unknown, and could be either presynaptic or post-synaptic. We have not investigated co-operativity in the ganglion.

Role of l.t.p. in vivo

Our studies were not designed to prove a physiological role for l.t.p. However, it is reasonable to consider whether l.t.p. could be expected to occur in the ganglion *in vivo*. All of our experiments have been conducted *in vitro* and, in addition to the tissue preparation and use of an artificial bathing medium, we have typically imposed four other artificial conditions: (1) low temperature, which prevents development of an anoxic core; (2) addition of atropine; (3) addition of a nicotinic antagonist; (4) repetitive stimulation of all preganglionic fibres simultaneously. The studies presented here demonstrate that low temperature, atropine and a nicotinic antagonist provide for convenient quantification of l.t.p. but are not requisites for eliciting the phenomenon. Many of the experiments utilized tetani of 5 Hz for 20 s and, in seven out of seven ganglia, we found that l.t.p. could be induced by just twenty-five stimuli at 5 Hz. This is certainly within the range of activity *in vivo* (Skok, 1980; Janig, Sundlof & Wallin, 1983). It seems reasonable to hypothesize that l.t.p. may occur in the *in vivo* sympathetic ganglion as a consequence of heightened preganglionic activity. Further studies are needed to test this hypothesis and to examine the role of l.t.p. in the autonomic nervous system.

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