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POST-TETANIC DEPOLARIZATION IN SYMPATHETIC NEURONES OF THE GUINEA-PIG

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SUMMARY

1. Repetitive intracellular stimulation at a frequency of 5–30 Hz for 1–10 s evoked in neurones of the isolated inferior mesenteric and superior cervical ganglia of the guinea-pig three types of post-spike membrane potential changes: (i) hyperpolarization, (ii) hyperpolarization followed by a slow depolarization, and (iii) a second hyperpolarization following the initial two responses.

2. The initial post-spike hyperpolarization had a mean duration of 2·0 s and was often associated with a fall in membrane resistance; it could be elicited in every sympathetic neurone studied. This response was termed the post-tetanic hyperpolarization (PTH).

3. The slow depolarization which could be induced only in a portion of neurones had a mean amplitude and duration of 2·2 mV and 27·5 s, respectively; it was termed the post-tetanic depolarization (PTD).

4. PTD was associated with a fall in membrane resistance, augmented by membrane hyperpolarization, and reduced by depolarization; its mean extrapolated equilibrium potential was −38 mV.

5. PTD was not blocked by nicotinic and muscarinic antagonists, or α-and β-adrenergic receptor antagonists, whereas it was suppressed by adrenaline, noradrenaline, Co^{2+} and a low Ca^{2+} solution.

6. The amplitude of the single spike after-hyperpolarization in normal Krebs solution as well as in high K^+ solution was increased during PTD; furthermore, conditioning hyperpolarization to the level of E_K increased the amplitude of PTD in normal Krebs as well as in high K^+ solution.

7. PTD with similar amplitude, time course and membrane characteristics could be evoked in a portion of neurones of the rabbit superior cervical ganglia; however, PTD was not detected in neurones of the rat superior cervical ganglia.

8. Decentralization of the guinea-pig and rabbit superior cervical ganglia for 14 d did not alter the number of neurones in which PTD could be elicited, its amplitude, or its time course.

9. Our results suggest that a chemical substance(s) is responsible for the generation of PTD; it may be released from the soma and/or dendrites and acts in an auto-receptive manner on the cells in question. The nature and origin of the second hyperpolarization remain to be clarified.

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INTRODUCTION

That direct or antidromic repetitive stimulation elicits in a number of excitable tissues a post-spike hyperpolarization termed the post-tetanic hyperpolarization (PTH) is well documented (Ritchie & Straub, 1957; Straub, 1961; Nakajima & Takahashi, 1966; Kuno, Miyahara & Weakly, 1970; Brodwick & Junge, 1972; Thomas, 1972; Jansen & Nicholls, 1973). The PTH exhibited by the bullfrog paravertebrate ganglia was analysed in detail and found to consist of two components: an early and late phase which differ in their time course and ionic mechanisms (Koketsu, 1971; Minota, 1974). The duration of early phase of PTH was relatively short (< 15 s) as compared to that of the late phase which may last for minutes (Minota, 1974).

On the other hand, the post-spike membrane potential change in mammalian sympathetic neurones induced by repetitive cell activation has not been analysed as yet; this analysis is the subject of the present investigation. It appears that post-spike activity in mammalian sympathetic neurones that follows a train of action potentials is distinctively different from that of amphibian neurones; furthermore, it may represent a novel type of neuronal response that has not been described heretofore in other excitable cells. The depolarizing phase of the response found in this study should be referred to as post-tetanic depolarization (PTD) to distinguish it from the slow and late slow excitatory post-synaptic potentials elicited by repetitive preganglionic nerve stimulation (Nishi, 1974; Kuba & Koketsu, 1978; Dun, 1980).

METHODS

The superior cervical ganglia of adult male guinea-pigs (250–300 g), rabbits (1·5–2·0 kg) and rats (200–250 g), and the inferior mesenteric ganglia of the guinea-pigs were rapidly excised from animals anaesthetized with pentobarbital sodium (30 mg/kg, I.P.). The ganglia were transferred to the recording chamber and perfused continuously with a Krebs solution of the following composition (in mmol/l): NaCl, 117; KCl, 4·7; $CaCl_2$, 2·5; $MgCl_2$, 1·2; $NaHCO_3$, 25; NaH_2PO_4, 1·2; glucose, 11·5. The solution was equilibrated with 95% O_2 and 5% CO_2; bath temperature was maintained at 36–37 °C. In experiments in which low Ca^{2+} (0·25 mmol/l) and high Mg^{2+} (12 mmol/l) solution was used to perfuse the ganglia, the content of NaCl in Krebs solution was proportionally reduced.

Intracellular recordings from neurones of isolated sympathetic ganglia were obtained using fibre-containing glass micro-electrodes, filled with KCl (3 mol/l) and having a resistance of 30–50 MΩ. Repetitive spike potentials were elicited by the passage of depolarizing current pulses through the intracellular micro-electrode utilizing a bridge circuit of the preamplifier (WPI 701). The input resistance was calculated from the voltage changes produced by the passage of repetitive hyperpolarizing current pulses (100–500 ms duration) at a frequency of 0·3–0·5 Hz. Voltage and current recordings were displayed on a Gould Brush Pen recorder (model 2200) and a Tektronix oscilloscope. Figures were reproduced from the tracings of the pen recorder.

The procedure used for decentralization of the superior cervical ganglia of the rabbit and guinea-pig has been described previously (Dun, Nishi & Karczmar, 1976). Rabbits and guinea-pigs were anaesthetized with pentobarbitone, and a 1–2 cm segment of the right cervical sympathetic trunk was removed under aseptic precautions. Success of the surgical denervation was evident in the course of 3–4 d by the occurrence of miosis and ptosis on the operated as compared with the unoperated contralateral side (for further information, see Dun *et al.* 1976).

The following compounds were used: adrenaline bitartrate, atropine sulphate, hexamethonium bromide, noradrenaline bitartrate, phenoxybenzamine hydrochloride, propranolol hydrochloride and D-tubocurarine chloride.

RESULTS

Types of post-spike membrane potential changes

Single intracellular stimulation of the neurones of the inferior mesenteric and superior cervical ganglia of the guinea-pigs elicited a spike discharge which was followed by an after-hyperpolarization lasting from one to several hundred milliseconds. When a train of depolarizing current pulses (5–30 Hz) was applied to sympathetic neurones for 1–10 s, three types of post-spike membrane potential change could be distinguished.

The membrane potential change of the first type consisted of a single phase of hyperpolarization that lasted for a few seconds following the spike potentials; this

TABLE 1. Incidence of sympathetic neurones in three species that exhibited three types of post-spike membrane potential change

	No. of cells	PTH	PTD	Second hyperpolarization
Guinea-pig				
SCG	20	20	13	1
IMG	38	38	24	3
Rabbit SCG	22	22	14	1
Rat SCG	18	18	0	0
Decentralized				
Rabbit SCG	9	9	5	0
Guinea-pig SCG	14	14	8	1

Abbreviations: post-tetanic hyperpolarization (PTH); post-tetanic depolarization (PTD); inferior mesenteric ganglia (IMG); superior cervical ganglia (SCG).

response represented the post-tetanic hyperpolarization (PTH). The amplitude of PTH varied markedly from cell to cell as it ranged from 2 to 15 mV in neurones exhibiting resting membrane potentials of −50 to −60 mV. The duration of PTH also showed considerable variation, ranging from 1 to 3 s; the mean was 2·0 ± 0·8 s ($n = 16$, mean ± S.D.). It should be emphasized that PTH was observed in every sympathetic neurone of the three species studied (Table 1), and that it was often associated with a decrease in membrane resistance (see below, and Figs. 3 and 7).

The second type of post-spike membrane activity consisted of a biphasic membrane potential change, as the initial PTH was followed by a slow post-tetanic depolarization (PTD). About 60 % of the neurones in guinea-pig sympathetic ganglia exhibited this biphasic response (Table 1). The mean amplitude and duration of PTD in fifty-eight guinea-pig sympathetic neurones was 2·2 mV and 27·5 s, respectively. One of the representative recordings is shown in Fig. 1*A*.

The third type of post-spike activity following repetitive cell activation was characterized by a triphasic membrane potential change, i.e. the PTH and the PTD were succeeded by a second, long-lasting hyperpolarization (Figs. 1*B* and 3). The number of cells that exhibited this type of response in guinea-pig or in other sympathetic ganglia was small (Table 1). The time course of the second hyperpola-

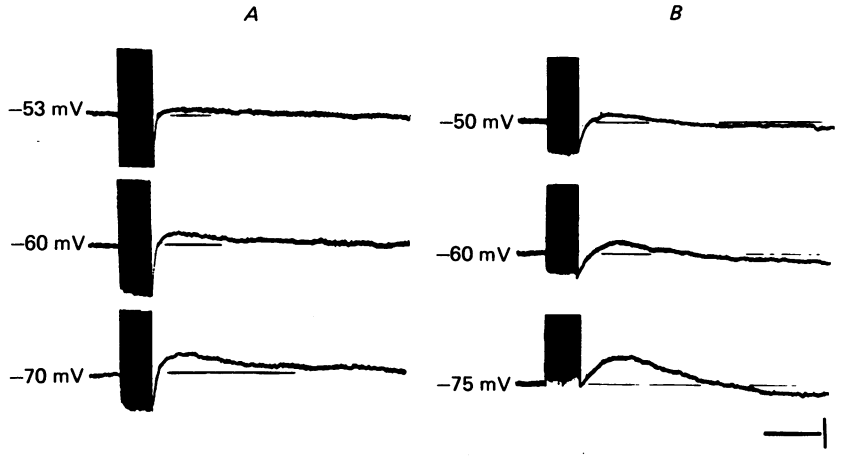

Fig. 1. Post-tetanic membrane potential change recorded from two different guinea-pig inferior mesenteric ganglion cells. Repetitive depolarizing current pulses (30 Hz) were delivered for 10 s to the neurones through the intracellularly placed micro-electrodes. In cell *A*, PTH was followed only by a PTD; increasing the membrane potential from the resting level of −53 mV to −60 mV and −70 mV augmented PTD. In cell *B*, PTD was followed by a second hyperpolarization lasting more than 80 s (entire time course not shown); conditioning hyperpolarization enhanced the amplitude of PTD as well as that of the second hyperpolarization. The peaks of spikes in these and in all subsequent recording were attenuated. Calibration: 10 mV and 20 s.

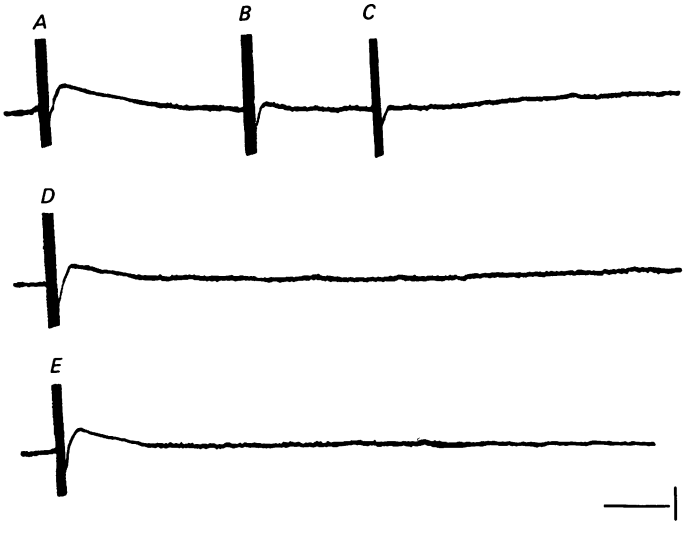

Fig. 2. Relation between the amplitude of PTD and stimulation interval in a guinea-pig inferior mesenteric ganglion cell. Repetitive intracellular stimulation (15 Hz) was applied to the neurone for 5 s. Records *A–C* constitute a continuous tracing; records *D* and *E* were obtained 10 min after the last preceding stimulation. Note that the amplitude of PTD declined successively in records *B* and *C* as the intervals between stimulation were shortened. When stimulus trains were applied at 10 min intervals, a relatively constant PTD could be elicited as shown in records *D* and *E*. Calibration: 10 mV and 30 s.

rization was long lasting; it ranged from 40 to 180 s, with a mean of 98 s ($n = 6$, mean $\pm$ S.D.). The average amplitude of the second hyperpolarization was 3·0 mV as recorded at the resting membrane potentials of -50 to -60 mV.

It should be pointed out that the three types of the change occurred only following spike discharges of the neurones in question. Thus, intracellular application of depolarizing current pulses that were just below threshold level caused no post-spike membrane potential change even when applied at a frequency of more than 30 Hz.

The amplitude of PTD was inversely related to the interval between successive periods of stimulation. This phenomenon was noticed at intervals of a few seconds to less than 5 min as shown in Fig. 2*A–C*. However, when the tetanic pulses were applied at intervals of 5–10 min, the amplitude of PTD showed no appreciable decrement (Fig. 2*D* and *E*). An interval of 5–10 min between each stimulation was sufficient to elicit a reproducible PTD in a given neurone.

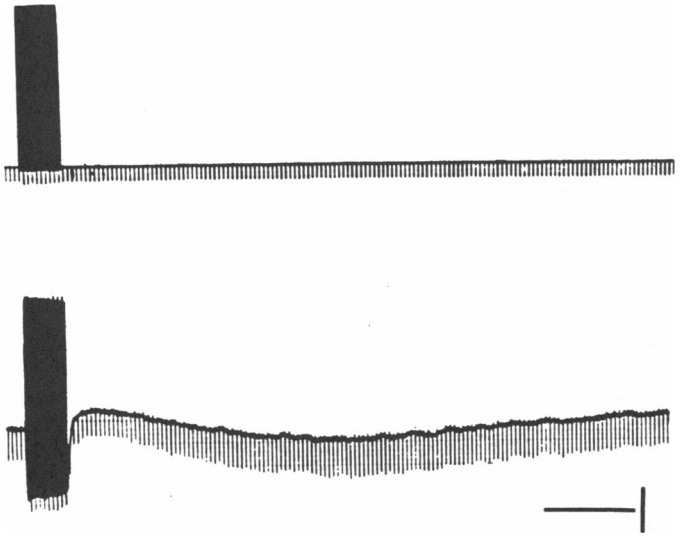

Fig. 3. Membrane resistance change during post-tetanic membrane potential change of a guinea-pig superior cervical ganglion cell. Anelectrotonic potentials (lower tracing) were induced by hyperpolarizing current pulses of 200 ms duration (upper tracing). Repetitive intracellular stimulation (20 Hz) was applied to the ganglion cell for 10 s. Note the decrease of membrane resistance during the course of PTH and PTD, and the increase of membrane resistance in the course of the second hyperpolarization. Calibration: 10 mV, 0·5 nA and 20 s.

Membrane resistance changes

Anelectrotonic potentials were used to estimate neuronal input resistance change during post-spike membrane potential changes. In the case of PTH, a decrease in membrane resistance was frequently observed in sympathetic neurones of the guinea-pig as well as in other two species studied (Figs. 3 and 7).

There was also a moderate decrease of membrane resistance during the generation of PTD in all twenty-four guinea-pig sympathetic neurones examined; the mean reduction amounted to 16·3 $\pm$ 9·2 % (mean $\pm$ S.D.), with a range of 9–33 %. In the

experiment shown in Fig. 3, the maximum reduction of membrane resistance at the peak of PTD was about 20%. The membrane resistance change associated with the second, long-lasting hyperpolarization could not be analysed statistically, as only a few neurones showed this type of response; an increase of 14% was noted in the case of the neurone shown in Fig. 3. Membrane resistance was monitored in five of the six neurones that exhibited the second phase of hyperpolarization; two exhibited moderate increase of resistance and no measurable change occurred in the remaining three neurones.

Conditioning polarization of the membrane

The relation between the amplitude of PTD and the membrane potential was investigated in this series of experiments. In all sixteen neurones examined, conditioning hyperpolarization consistently augmented the amplitude of PTD; two cells exhibiting this phenomenon are shown in Fig. 1. It is interesting to note that in the case of the neurone exhibiting a biphasic hyperpolarization pattern following the tetanic stimulation, the amplitude of the second hyperpolarizing response was also increased upon membrane hyperpolarization (Fig. 1*B*). No detailed study was made of the relationship between the response of the second hyperpolarization and the membrane potential.

The mean extrapolated equilibrium potential for PTD in twelve neurones which showed only PTD with no subsequent hyperpolarization was $-38 \pm 6{\cdot}2$ mV.

After-hyperpolarization and the effect of high K^+

The level of after-hyperpolarization following a single spike potential in autonomic neurones appears to be the potassium equilibrium potential (E_K, see Skok, 1973). The amplitude of after-hyperpolarization of the single spike increased invariably during PTD in all nine neurones studied. One of such experiments is illustrated in Fig. 4. In normal Krebs solution, the amplitude of after-hyperpolarization was increased by 37% at the peak of PTD (Fig. 4*A*); when the membrane potential was increased to the level at which the after-hyperpolarization was nearly nullified, the amplitude of PTD was augmented (Fig. 4*B*) as compared to that elicited at the resting membrane potential (Fig. 4*A*), and the after-hyperpolarization of single spike was, again, increased (Fig. 4*B*). When perfusing Krebs solution was changed to high K^+ (10 mmol/l) Krebs, the membrane depolarized slightly by about 5 mV; the depolarization was accompanied by a reduction of the amplitude of the single spike after-hyperpolarization (Fig. 4*C*). Under these conditions, the response of PTD was moderately depressed (Fig. 4*C*); the mean reduction was 23% ($n = 9$). When the membrane was hyperpolarized in high K^+ solution to the level at which the after-hyperpolarization of single spike was nullified, the PTD response was enhanced, and the single spike after-hyperpolarization during PTD was increased (Fig. 4*D*).

Effect of low Ca^{2+}/high Mg^{2+} solution, and of Co^{2+}

The effects of low Ca^{2+}/high Mg^{2+} solution on the PTD response was examined in seven neurones. Superfusion with Krebs solution containing low Ca^{2+} (0·25 mmol/l)/high Mg^{2+} (12 mmol/l) caused transient, slight membrane depolarization or hyperpolarization; the membrane potential gradually returned to near control

level after a few minutes of continuous superfusion. In all seven neurones examined, the PTD was either markedly diminished or abolished in the Ca^{2+}-deficient solution; one of the pertinent experiments is depicted in Fig. 5. As can be seen the PTD was eliminated following the superfusion of the ganglion with low Ca^{2+} solution for about 7 min (Fig. 5*A* and *B*); furthermore, conditioning hyperpolarization of the membrane revealed no evidence of PTD (Fig. 5*C*). The response recovered fully following a period of 5 min wash with normal Krebs solution (Fig. 5*D*).

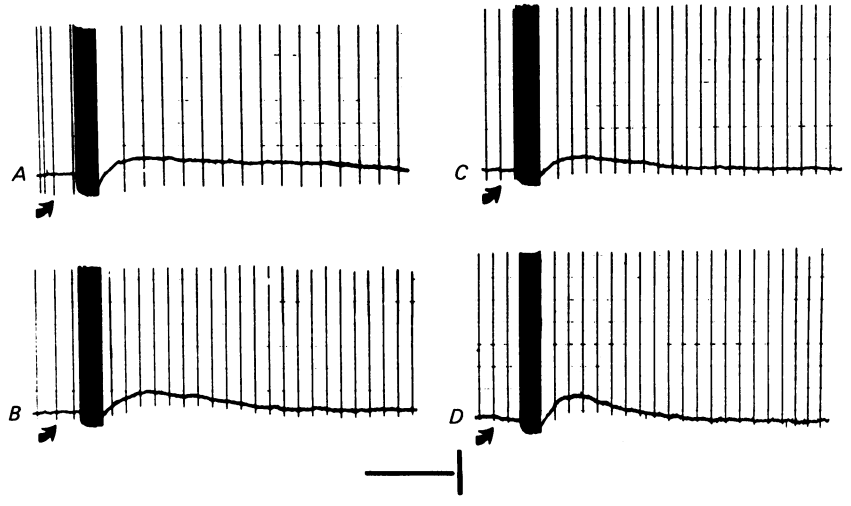

Fig. 4. Effect of conditioning hyperpolarization to the level of E_K, and of high K^+ (10 mmol/l) on the after-hyperpolarization of single spikes and on PTD response in a guinea-pig inferior mesenteric ganglion cell. PTD was elicited by a train of depolarizing current pulses (20 Hz for 10 s). Arrows denote after-hyperpolarization following a single spike discharge. The responses in records *A* and *B* were elicited in normal Krebs solution; records *C* and *D* were obtained in high K^+ solution. *A* and *B*, PTD elicited at resting membrane potential of −56 mV and at the membrane potential level at which the after-hyperpolarization was nearly nullified, respectively. *C* and *D*, PTD evoked at membrane potential of −52 mV and at the level at which the after-hyperpolarization was nullified in high K^+ solution. Calibration: 10 mV and 20 s.

The effect of Co^{2+} (2 mmol/l) on PTD was examined in an additional three neurones. The amplitude of PTD was substantially decreased by Co^{2+} in all three neurones; the mean reduction amounted to 72%. The depressant effect of Co^{2+} was reversible after washing with normal Krebs solution.

Pharmacology of PTD

The amplitude or duration of PTD was not altered by nicotinic (D-tubocurarine, 0·1 mmol/l or hexamethonium, 0·5 mmol/l) and muscarinic (atropine, 1 μmol/l) antagonists in any of the eight neurones studied. Similarly, PTD was not appreciably affected by α-adrenergic (phenoxybenzamine, 10 μmol/l) and β-adrenergic (propranolol, 5 μmol/l) receptor antagonists in four neurones tested.

On the other hand, adrenaline and noradrenaline in concentrations of 1–10 μmol/l consistently and reversibly suppressed PTD. In the experiment shown in Fig. 6,

adrenaline (10 μmol/l) reduced the amplitude of PTD to about 30 % of control in less than 5 min (Fig. 6*B*); the effect was readily reversible upon wash with drug-free Krebs solution (Fig. 6*C*). The average reduction of PTD induced by 1 and 10 μmol/l concentrations of adrenaline was 45 % ($n = 4$) and 67 % ($n = 3$), respectively. It should be pointed out that when employed at these concentrations, catecholamines did not affect the membrane potential of the sympathetic neurones in a consistent manner; there was either no change or slight hyperpolarization and depolarization were observed.

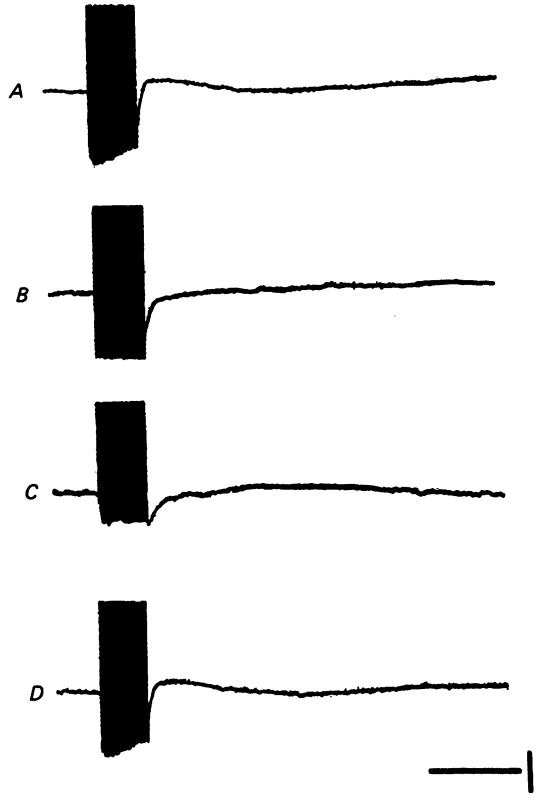

Fig. 5. Effect of low Ca^{2+} (0·25 mmol/l)/high Mg^{2+} (12 mmol/l) on the PTD in a guinea-pig inferior mesenteric ganglion cell. PTD was evoked by a train of depolarizing current pulses (10 Hz for 10 s). *A*, PTD evoked at the resting membrane potential of −52 mV. *B* and *C* are recordings taken 7 and 10 min after superfusing the ganglion with low Ca^{2+} solution at membrane potentials of −52 and −62 mV, respectively. Note that tetanic stimulation elicited only PTH in *B* and *C*. *D*, 5 min after superfusion in normal Krebs solution, PTD was induced again at the membrane potential of −52 mV. Calibration: 10 mV and 20 s.

Rabbit and rat sympathetic ganglia

All sympathetic neurones of rabbit and rat superior cervical ganglia that were tested exhibited PTH (Table 1).

PTD similar in amplitude, time course and membrane characteristics to that of the guinea-pig sympathetic neurones could be elicited in fourteen of the twenty-two

rabbit superior cervical ganglion cells tested (Table 1); the mean amplitude and duration of the PTD were 1·8 mV and 31·2 s, respectively. Moreover, the PTD response recorded from four different neurones was uniformly sensitive to the blocking effect of low Ca^{2+} solution.

On the other hand, no measurable PTD was observed in any of the eighteen neurones sampled from five superior cervical ganglia of the rat. Thus, in the case of the neurone illustrated in Fig. 7, repetitive intracellular stimulation elicited only the PTH (Fig. 7*A*), and conditioning hyperpolarization (to −60 mV) did not unmask the presence of PTD (Fig. 7*A*); the membrane resistance was markedly reduced during the PTH (Fig. 7*B*).

Fig. 6. Effect of adrenaline (10 μmol/l) on the PTD in a guinea-pig inferior mesenteric ganglion cell. PTD was elicited by a train of depolarizing current pulses (30 Hz for 10 s). *A*, control response; *B*, 4 min after adrenaline superfusion; *C*, 10 min after wash with normal Krebs solution. Calibration: 10 mV and 20 s.

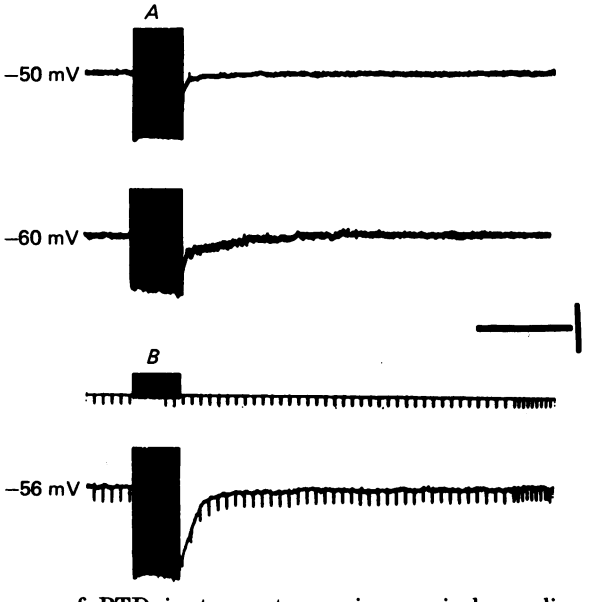

Fig. 7. Absence of PTD in two rat superior cervical ganglion cells. *A*, repetitive intracellular stimulation at a frequency of 30 Hz for 10 s elicited only PTH at the membrane potential levels of −50 and −60 mV. *B*, anelectrotonic potentials (lower tracing) were induced by hyperpolarizing current pulses of 100 ms duration (upper tracing). Note the marked decrease in membrane resistance during PTH. Recordings in *A* and *B* were taken from two different cells. Calibration: 10 mV, 0·5 nA and 20 s.

Effect of decentralization

In two rabbits and two guinea-pigs, the superior cervical ganglia were chronically decentralized for 14 d by surgical removal of a segment of the cervical sympathetic nerve trunks (Dun *et al.* 1976). PTD could be elicited from five of the nine neurones tested of the decentralized rabbit superior cervical ganglia; the figures for the guinea pig were eight out of fourteen (Table 1). The mean amplitude and duration of PTD from thirteen responsive neurones was 1·3 mV and 26 s, respectively. Furthermore, low Ca^{2+} solution effectively abolished PTD in all three neurones tested, and adrenaline (10 μmol/l) strongly suppressed the PTD in other two neurones.

DISCUSSION

This study shows that the membrane potential changes recorded in mammalian sympathetic neurones after repetitive spikes have been elicited differ in several respects from the post-tetanic changes described for other excitable cells. The most significant difference was that repetitive cell activation generated, in addition to the initial post-stimulus hyperpolarization (PTH), a depolarization of considerable duration, and, in a few cells, a second prolonged hyperpolarization.

The phenomenon of post-spike activity has been analysed in a variety of preparations, for example, myelinated (Straub, 1961) and unmyelinated (Ritchie & Straub, 1957) nerve fibres, leech ganglion cells (Baylor & Nicholls, 1969), crayfish stretch receptors (Nakajima & Takahashi, 1966), cat dorsal spinocerebellar tract neurones (Kuno *et al.* 1970), Aplysia giant neurones (Brodwick & Junge, 1972) and bullfrog sympathetic ganglion cells (Koketsu, 1971; Minota, 1974). In all these tissues, post-stimulus membrane activity consisted of a hyperpolarization which was not followed by depolarization. Moreover, it was demonstrated that PTH of these cells was an after-potential, there being no involvement of chemical transmission process. The PTH in question appeared to be due to either of two mechanisms or their combination: (i) activation of a metabolic pump, and (ii) conductance change. For example, the early phase of PTH in bullfrog sympathetic neurones seems to be generated by an electrogenic sodium pump, whereas the late component may be due to an increase of a calcium-sensitive potassium conductance (Koketsu, 1971; Minota, 1974).

The initial hyperpolarization observed in mammalian sympathetic neurones was initiated only after spike discharge and it was not eliminated by a low Ca^{2+} solution or catecholamines; this suggests that it does not depend on the release of a transmitter. Altogether, this phase of the post-tetanic response of the mammalian sympathetic neurones seems to be analogous to the PTH found in other excitable cells. The ionic mechanism underlying PTH in mammalian sympathetic neurones was not studied in detail; the finding that PTH was frequently accompanied by a reduction in membrane resistance suggests that it may result from an increase of G_K as does the PTH of Aplysia giant neurones (Brodwick & Junge, 1972) and the late component of the PTH of the bullfrog sympathetic neurones (Minota, 1974).

The significant finding of the present study is the demonstration of novel phenomena of post-stimulus membrane potential changes in mammalian sympathetic

ganglion cells. The information available with respect to the delayed hyperpolarization that was observed in a few neurones following the PTD is at this time quite scanty. On the other hand, the PTD proved to be a consistent membrane response that could be studied in some detail. It should be emphasized that in contrast to the slow excitatory and late slow excitatory post-synaptic potentials that are elicited by preganglionic nerve stimulation (Nishi, 1974; Kuba & Koketsu, 1978; Dun, 1980), PTD was evoked by direct intracellular stimulation.

An important question which needs to be resolved is whether or not PTD is an after-potential following repetitive cell activation. In this context, the possibility that the depolarization may result from an accumulation of extracellular K ions should be considered. Several lines of evidence obtained in this study indicate that PTD cannot be explained satisfactorily as due to accumulation of K ions. Firstly, repetitive antidromic stimulation of giant synapses of the squid leads to accumulation of K ions; the latter causes a reduction of after-hyperpolarization of the single spike potential (Erulkar & Weight, 1977). Yet, the amplitude of after-hyperpolarization subsequent to a single spike was not decreased during PTD, and in fact, it was increased in many instances proportionally to the magnitude of membrane depolarization. Secondly, hyperpolarization of the membrane to the level of E_K should markedly reduce or nullify PTD if it were due to an accumulation of K ions. Yet conditioning hyperpolarization enhanced, rather than depressed the PTD; moreover, this effect was observed even in high K^+ solution in which the E_K was shifted closer to the membrane potential. Lastly, if PTD were to be generated by an accumulation of K ions, it should be detectable in every neurone. This was not the present finding; in fact, the number of neurones that exhibited PTD in a given ganglion appeared to be species dependent; it was observed in a large portion of neurones in the guinea-pig and rabbit sympathetic ganglia, but was not detected in the rat sympathetic ganglion cells. This latter finding should not be, however, regarded as conclusive, as the number of responsive neurones in these ganglia may have been small; these neurones may have been missed during random sampling with intracellular microelectrodes. Alternatively, the PTD of these neurones may have been small and undetectable.

Our results, on the other hand, suggest that a chemical substance may be responsible for a change in the permeability to certain ions of the soma and/or dendrites, and the generation of PTD. This hypothesis explains the findings that membrane resistance was decreased during the PTD, and that its equilibrium potential was more positive than the resting membrane potential. The concept that PTD is generated via a transmitter-like substance is also consistent with the blockade of PTD by agents that are known to depress transmitter release, i.e. low Ca^{2+} solution, Co^{2+} and catecholamines. Adrenaline and noradrenaline have been shown to inhibit release of transmitter from a number of synapses, including the liberation of acetylcholine (ACh) from sympathetic ganglia (Christ & Nishi, 1971; Dun & Nishi, 1974; Dun & Karczmar, 1977).

The origin and nature of the substance or substances responsible for PTD is of interest, and remains to be clarified. As the neurones in the present study were activated directly by intracellular stimulation, the substance in question may have been released from the cell body or dendrites rather than from preganglionic fibres. Moreover, the finding that chronic preganglionic denervation caused no apparent

change in the number of neurones which could generate the PTD, or in the characteristics of the depolarization supports the notion that the substance may be released from the soma and/or dendrites of the cell and acts in an autoreceptive manner on the soma and/or dendrites of the cell in question. In this context, our recent study showed that dendrites of mammalian sympathetic neurones exhibit varicosities and that they may form dendro-dendritic and dendro-somatic synapses (Kondo, Dun & Pappas, 1980). It is also noteworthy that this form of autoreceptive interaction has been suggested to occur in certain central synapses; for example, dopamine released from dendrites of dopaminergic neurones in substantia nigra may act on the 'autoreceptors' of the same neurones from which it is released or it may affect neighbouring neurones (Geffen, Jessell, Cuello & Iversen, 1976; Korf, Zielman & Westerink, 1976).

If indeed the PTD is generated by a transmitter-like substance, what is its nature? ACh appears not to be involved as nicotinic and muscarinic antagonists employed in concentrations sufficient to block cholinergic responses of the sympathetic ganglia (Dun *et al.* 1976) did not modify PTD. Similarly, adrenaline or noradrenaline are not likely to be the candidates as PTD was not affected significantly by α- and β-adrenergic receptor antagonists. It appears pertinent to point out that autonomic neurones contain several transmitters, or transmitter-like substances (Hökfelt, Johansson, Ljungdahl, Lundberg & Schultzberg, 1980). For example, somatostatin or a closely related peptide co-exists in noradrenaline-containing neurones in sympathetic ganglia (Hökfelt, Elfvin, Elde, Schultzberg, Goldstein & Luft, 1977), and vasoactive intestinal polypeptide or a related peptide is present in cholinergic autonomic neurones (Lundberg, Hökfelt, Schultzberg, Uvnäs-Wallensten, Kohler & Said, 1979). The physiological function of PTD in mammalian sympathetic neurones also remains to be studied. The significance of PTH in the transfer of neuronal signals is that it may serve as a 'self-biasing' device in regulating the membrane excitability for a period of seconds to minutes. PTD may thus represent an effective cellular mechanism involved in counteracting PTH under circumstances yet to be defined. It is noteworthy in this regard that the duration of PTH in mammalian sympathetic neurones is considerably shorter than that reported for other excitable cells (for example see Minota, 1974).

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