J. Physiol. (1984), **351**, *pp.* 583–593
With 7 text-figures
Printed in Great Britain

SLOW EXCITATORY POST-SYNAPTIC CURRENTS IN BULL-FROG SYMPATHETIC NEURONES

BY T. AKASU, J. P. GALLAGHER*, K. KOKETSU AND PATRICIA SHINNICK-GALLAGHER*

*From the Department of Physiology, Kurume University School of Medicine, 67 Asahi-machi, Kurume 830, Japan and *Department of Pharmacology and Toxicology, The University of Texas Medical Branch, Galveston TX 77550, U.S.A.*

(*Received 1 November 1983*)

SUMMARY

1. Electrogenesis of the slow excitatory post-synaptic current (slow e.p.s.c.) was analysed with voltage-clamp methods in curarized sympathetic ganglion cells of bull-frogs. Three types of slow e.p.s.c. were observed from B neurones of sympathetic ganglia.

2. The type I slow e.p.s.c. was associated with a decrease in membrane conductance, was depressed by membrane hyperpolarization and nullified at −60 to −70 mV. It was observed in 65 % of the sympathetic neurones studied.

3. The type II slow e.p.s.c. was associated with an increase in membrane conductance, was depressed by membrane depolarization and nullified at around +5 mV. It was observed in 14 % of the neurones studied.

4. A third type of slow e.p.s.c. was recorded from 21 % of the sympathetic neurones in this study. This slow e.p.s.c. was a mixed type having characteristics of both type I and type II slow e.p.s.c.s.

5. Activation of muscarinic cholinergic receptors by application of acetylcholine (ACh) also produced two types of inward currents. The nature of each type of muscarinic slow ACh current was similar to that of each type of slow e.p.s.c.

6. The time course of the falling phase of type I and type II slow e.p.s.c.s was dependent on the membrane potential.

7. The type I slow e.p.s.c. was primarily dependent on extracellular K^+ and appeared to be produced by a suppression of the M-current (Brown & Adams, 1980). The type II slow e.p.s.c. was due to an increased conductance, probably to Na^+, and other cations.

INTRODUCTION

Stimulation of the sympathetic chain at the level of the 7th ganglion evokes two different types of excitatory post-synaptic potential (e.p.s.p.) in bull-frog sympathetic ganglia, that is, a fast e.p.s.p. and a slow e.p.s.p. which are mediated by acetylcholine (ACh). The fast e.p.s.p. is due to activation of the nicotinic receptors (Nishi & Koketsu, 1960) and the slow e.p.s.p. is due to activation of muscarinic receptors (Koketsu, 1969).

Nishi, Soeda & Koketsu (1969) classified the slow e.p.s.p. of bull-frog sympathetic ganglia into three different types according to the effect of membrane polarization on the slow e.p.s.p. recorded from a particular neurone. Weight & Votava (1970) proposed that there was only one type of slow e.p.s.p. which was produced by a decrease of the K^+ conductance (G_K) of the post-ganglionic cell. On the other hand, Kuba & Koketsu (1976, 1978), using current-clamp techniques, further analysed in detail the ionic mechanism of the slow e.p.s.p. They concluded that the slow e.p.s.p. is generated not only by a decrease in K^+ conductance but also by increases in Na^+ and Ca^{2+} conductances.

Brown & Adams (1980), using voltage-clamp techniques, identified a voltage-dependent K^+ current (M-current) different from the delayed rectifier K^+ current. This M-current was suppressed by the muscarinic agonist, muscarine, and during the generation of the slow excitatory post-synaptic current (e.p.s.c.). They concluded that the slow e.p.s.c. was due to the selective inhibition of the M-current (Adams & Brown, 1982; Adams, Brown & Constanti, 1982). Recently, Kuffler & Sejnowski (1983), using a single-electrode voltage clamp, reported a slow e.p.s.p. associated with an increase in membrane conductance at hyperpolarized levels. This occurred in about half the neurones in addition to conductance decreases observed during muscarinic e.p.s.c.s at resting levels. We now report a detailed analysis of the types of slow e.p.s.c.s observed under two-electrode voltage-clamp conditions, namely a type I slow e.p.s.c. which is generated by a decrease in K^+ conductance, and a type II slow e.p.s.c. which is generated by an increase in cationic conductance, mostly a Na^+ conductance. Mixed types of slow e.p.s.c.s were also observed.

METHODS

Sympathetic ganglion chains were isolated from bull-frogs (*Rana catesbeiana*) weighing 300–400 g. The 8th or 9th ganglion was superfused in a small chamber with Ringer solution.

The method used for intracellular recordings with a single micro-electrode was similar to that described in a previous paper (Nishi & Koketsu, 1960). The voltage-clamp techniques were essentially the same as described elsewhere (Akasu & Koketsu, 1981). Cells were impaled with two electrodes, one for recording membrane potential filled with 3 M-KCl and the other one for injecting feed-back current filled with 1 M-K citrate. Electrodes were used which had tip resistances of 30–70 MΩ for recording membrane potentials and 20–30 MΩ for current injection. The feed-back amplifier was a Nihon Kohden CEZ-1100 with maximum gain of 10000. The feed-back current was monitored by a current–voltage converter (mounted in CEZ-1100) connected to a Ag–AgCl electrode in the bath. No residual fraction of the post-synaptic potentials remained during voltage clamping.

The Ringer solution contained (mM): NaCl, 112; KCl, 2·0; $CaCl_2$, 1·8 and $NaHCO_3$, 2·4. In some experiments, NaCl and $NaHCO_3$ were totally replaced by choline chloride, and the pH was adjusted to 7·2 with Tris-(hydroxymethyl) aminomethane chloride.

The ganglion cells were treated with (+)-tubocurarine (curare; 30 μM) to block completely the fast e.p.s.p. The slow e.p.s.p. was induced by a train of supramaximal preganglionic stimuli (30 Hz for 3–10 s) which were applied at the level of the 7th ganglion. This means that preganglionic B fibres should have been stimulated (Nishi, Soeda & Koketsu, 1965) yielding a slow e.p.s.p. without a contaminating slow inhibitory post-synaptic potential (slow i.p.s.p.) (Libet, Chichibu & Tosaka, 1968; Tosaka, Chichibu & Libet, 1968; Skok, 1973). All experiments were carried out at room temperature (20–26 °C).

The drugs used were acetylcholine chloride (Wako), (+)-tubocurarine chloride (Sigma) and atropine sulphate (Wako).

RESULTS

Conductance changes of the slow e.p.s.c.

Slow e.p.s.p.s were recorded from post-ganglionic neurones superfused with Ringer solution containing 30 μM-curare when a train of repetitive stimuli (40 Hz for 2–4 s) was applied to preganglionic B nerve fibres (Fig. 1). Two types of slow e.p.s.p.s could be identified based on changes in membrane resistance, as had been reported by Kuba & Koketsu (1976, 1978). An example of slow e.p.s.p.s which are associated with an increase (*A*) and a decrease (*B*) in membrane resistance is depicted in Fig. 1*a*. The

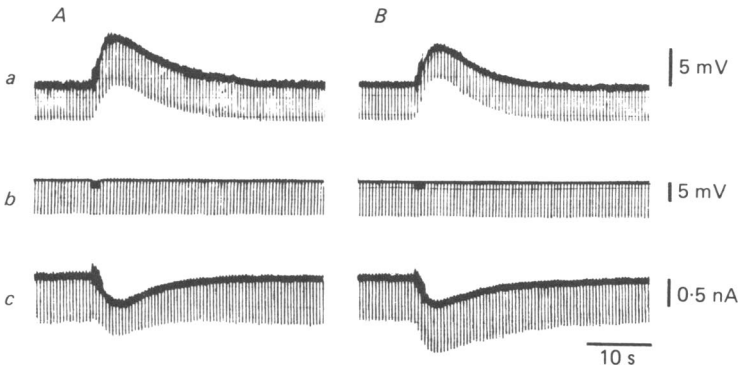

Fig. 1. Two types of slow e.p.s.p.s (*a*) and slow e.p.s.c.s (*c*) recorded in two different neurones, *A* and *B*. Slow e.p.s.p.s and slow e.p.s.c.s elicited by a train of supramaximal preganglionic stimuli 40 Hz (3–10 s) were recorded from the same neurones in *A* and in *B*. In record *a* the slow e.p.s.p.s were associated with increased (*A*) and decreased (*B*) membrane resistance. Vertical lines indicate the electrotonic pulses (duration: 100 ms) applied at 2 Hz in response to 0·5 nA of current injected into the neurone. Record *b* indicates the membrane potential held at −55 mV. Current pulses induced by voltage step commands from −55 to −65 mV were superimposed during the slow e.p.s.c.s. Records shown in *c* were two types of slow e.p.s.c. with decreased (*A*) and increased (*B*) membrane conductances.

conductance changes mediating these types of slow e.p.s.p. were examined in sympathetic neurones using voltage-clamp recordings. The resting membrane potentials of the ganglion cells penetrated with two micro-electrodes were $-53{\cdot}2 \pm 7{\cdot}3$ mV ($n = 34$) in a Ringer solution containing curare (30 μM). The slow e.p.s.c.s were induced by repetitive preganglionic B nerve stimulation (40 Hz for 2–5 s) in voltage-clamped sympathetic ganglion cells. Typical examples of slow e.p.s.c. responses demonstrating a conductance decrease (type I: *A*) and a conductance increase (type II: *B*) are illustrated in Fig. 1*c*. The type I slow e.p.s.c. induced at the resting potential level (−55 mV) was associated with a reduction of membrane conductance. This was determined from the decrease in current pulses in response to the step commands from −55 to −65 mV (Fig. 1*Ac*). The slow current relaxation (M-current) (Brown & Adams, 1980) was observed at potential levels more positive than −65 mV. The M-current was reduced during the type I slow e.p.s.p., confirming the finding of Adams & Brown (1982). The type II slow e.p.s.c. was recorded at

a membrane potential of −65 mV (Fig. 1*Bc*). During generation of the slow e.p.s.c. at −65 mV an increase in the membrane conductance was observed. Under this experimental condition, no current relaxation could be observed (cf. Fig. 2*B*).

Of forty-three voltage-clamped ganglion cells, twenty-eight cells (65%) exhibited a type I response and six cells (14%) showed a type II response. Nine cells (21%) had properties of both type I and type II responses. In the last group (mixed-type slow e.p.s.c.), the membrane conductance of the slow e.p.s.c. was increased as the membrane potential was hyperpolarized and decreased as the membrane potential was depolarized (cf. Fig. 5).

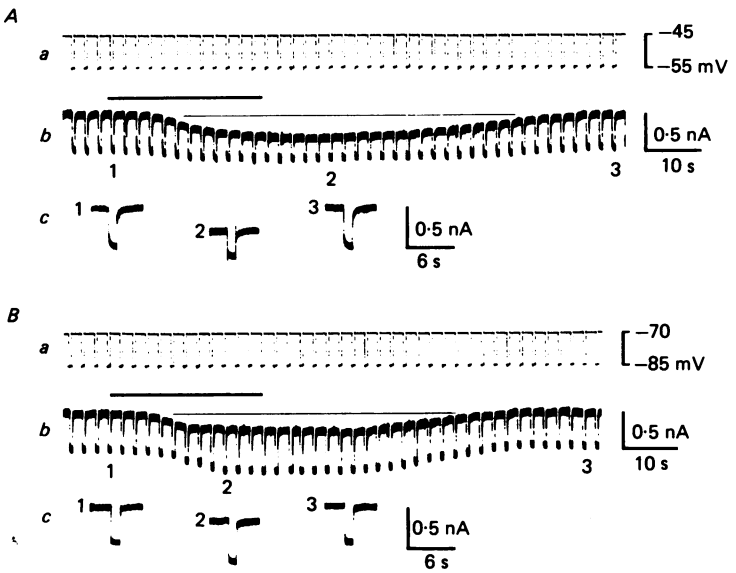

Fig. 2. Two types of slow muscarinic ACh currents with decreased (*A*) and increased (*B*) membrane conductances recorded in two different neurones. ACh (100 μM) was applied to the bath (a Ringer solution containing 30 μM-curare) marked by horizontal bars. *A* and *B*, record *a* indicates the membrane potential stepped from −45 to −55 mV in *A* and from −70 to −85 mV in *B* at a rate of 0·25 Hz, record *b* indicates ACh-induced membrane current superimposed with current pulses (duration: 1 s) in response to the step commands in *a*. Records shown in *c* were oscilloscope traces of current pulse recordings. Note that in *A*, the slow inward relaxation (record 1 and 3) was reduced by ACh (record 2). Records 1, 2 and 3 correspond to their respective numbers in record *b*.

Conductance changes of slow ACh current

Slow inward currents induced by the action of the ACh on muscarinic receptors were analysed at different holding potentials (Fig. 2). ACh (100 μM) added to the superfusing solution (a Ringer solution containing 30 μM-curare) induced the steady-state inward current (0·1–2 nA) in two different neurones, one held at a membrane potential of −45 mV (*A*) and the other at −70 mV (*B*). Two types of conductance change were observed during the generation of slow ACh currents. The membrane conductance was decreased (type I response) and increased (type II response). The M-current was reduced during the muscarinic action of ACh in a type I responding

neurone (Fig. 2*Ac*). We observed that the type II response could be recorded at a membrane potential of −70 mV (Fig. 2*Bc*), where the M-current was inactivated (Brown & Adams, 1980). Since the slow ACh current was completely eliminated by atropine (20 μM), the slow ACh current appeared to be identical to the slow e.p.s.c.

Effect of membrane potential on type I and type II slow e.p.s.c.

To examine the ionic mechanism of the type I and type II slow e.p.s.c.s, slow e.p.s.c.s were recorded at various membrane potential levels. As illustrated in Fig. 3, the type I slow e.p.s.c. was associated with a reduction of membrane conductance.

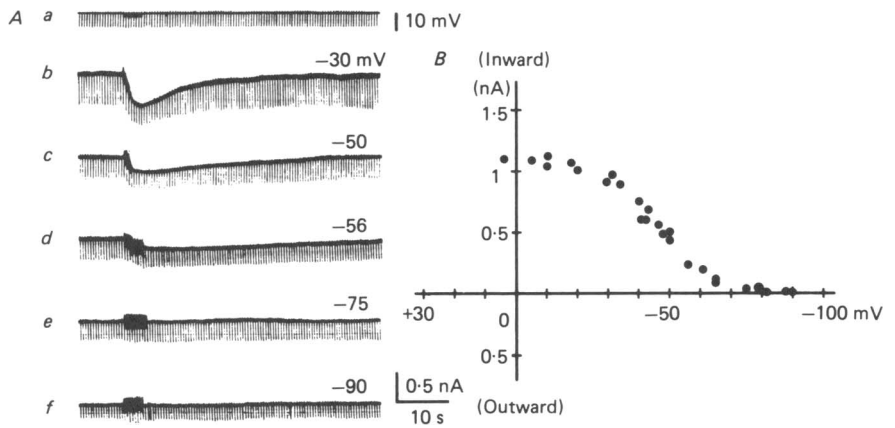

Fig. 3. The relationship between the amplitude of the type I slow e.p.s.c. and the holding potential level. *A*, the slow e.p.s.c. in response to supramaximal preganglionic stimuli (40 Hz for 4 s) was recorded at various holding levels between −30 and −90 mV. Command pulses (duration: 100 ms) of 10 mV were repeated at 2 Hz (top trace). *B*, peak amplitude of each slow e.p.s.c. response (ordinate) was plotted against the holding level (abscissa).

The behaviour of the type I slow e.p.s.c. and the underlying conductance changes appeared to be similar to a slow depolarization, and a slow e.p.s.p. generated by suppression of the M-current (Brown & Adams, 1980; Adams & Brown, 1982; Adams *et al.* 1982). The steady-state conductance was increased at depolarized membrane potential levels. Therefore, the change in conductance during the slow e.p.s.c. was readily apparent at depolarized potential levels (record *b* in Fig. 3*A*), while at membrane potential levels more negative than −60 mV, no obvious conductance change could be observed during the generation of the slow e.p.s.c. (records *e* and *f* in Fig. 3*A*). Fig. 3*B* illustrates the relationship between the amplitudes of slow e.p.s.c.s and holding membrane potentials (from −90 to +5 mV). The type I slow e.p.s.c. changed in amplitude at potential levels between −65 and −15 mV. The amplitude of the type I slow e.p.s.c. was increased by membrane depolarization and then reached a maximum level at a membrane potential of −10 mV. On the other hand, the amplitude of type I slow e.p.s.c. was decreased by conditioning hyperpolarizations and finally nullified at −75 mV; the slow e.p.s.c. did not reverse in polarity at more negative potential levels.

The type II slow e.p.s.c. differs characteristically from type I. The type II slow e.p.s.c. was associated with an increase of membrane conductance and an increase in amplitude as a result of conditioning hyperpolarizations (Fig. 4). At membrane potentials more negative than −70 mV, slow e.p.s.c.s were still observed and tended to be increased by more intense hyperpolarization. On the other hand, when the membrane was depolarized to levels more positive than −55 mV, the slow e.p.s.c. was depressed and finally nullified at +5 mV; reversal of type II slow e.p.s.c.

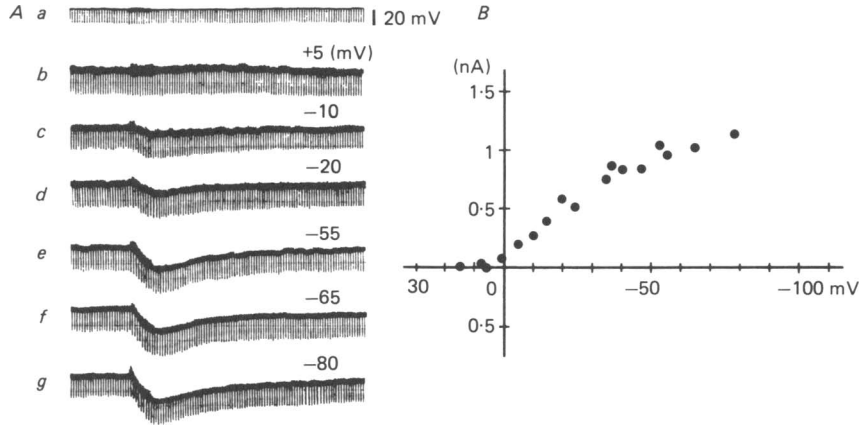

Fig. 4. The relationship between the amplitude of the type II slow e.p.s.c. and the holding potential level. *A*, the slow e.p.s.c.s were elicited by a train of repetitive preganglionic stimuli (40 Hz for 5 s) at various holding levels between +5 and −80 mV. Command pulses (duration: 100 ms) of 20 mV were repeated at 2 Hz (top trace). *B*, peak amplitude of each slow e.p.s.c. response (ordinate) was plotted against the holding level (abscissa).

was not observed. An increased membrane conductance was observed at potential levels between −10 and −80 mV, while no appreciable conductance change was observed at +5 mV. The mean null potential for the type II slow e.p.s.c.s recorded was $+6{\cdot}7 \pm 3{\cdot}2$ mV ($n = 6$).

Mixed type of slow e.p.s.c.

In some ganglion cells, slow e.p.s.c.s exhibited characteristics of both type I and type II responses. An example is shown in Fig. 5. At membrane potentials more positive than −55 mV, these cells showed preferentially a typical type I slow e.p.s.c. response, which was characterized by a decrease in conductance and increased amplitude as the membrane potential was further depolarized (Fig. 5*A*). This is the same relationship observed with type I slow e.p.s.c.s (cf. Fig. 3). At membrane potentials more negative than −55 mV, the properties of the slow e.p.s.c. changed, and showed those of typical type II responses, i.e. the amplitude of the slow e.p.s.c. was associated with an increase in conductance and a reverse relationship between the amplitude of the slow e.p.s.c. and holding membrane potential (Fig. 5*B*).

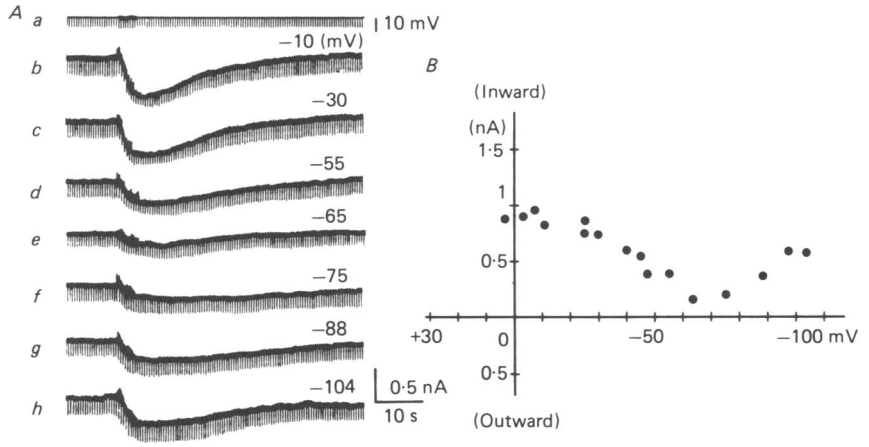

Fig. 5. The relationship between the amplitude of a mixed-type response and the holding potential level. *A*, the slow e.p.s.c.s were elicited by a train of repetitive preganglionic stimuli (40 Hz for 4 s) at various holding levels between −10 and −104 mV. Command pulses (duration: 100 ms) of 10 mV were repeated at 2 Hz (top trace). *B*, peak amplitude of each slow e.p.s.c. response (ordinate) was plotted against the holding level (abscissa).

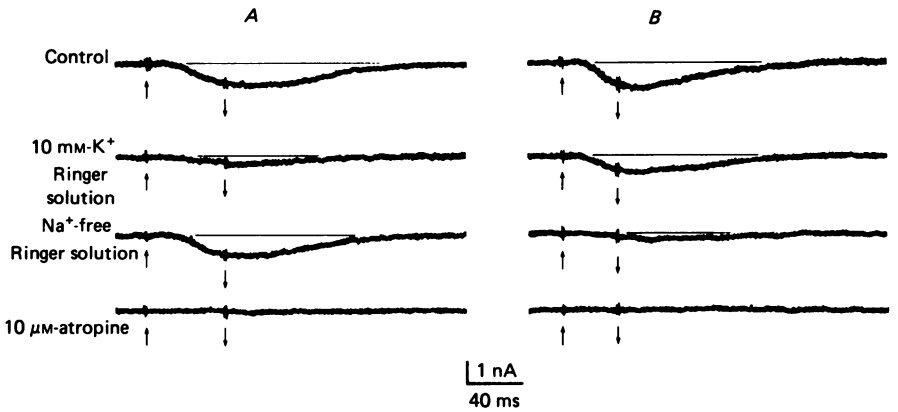

Fig. 6. The muscarinic ACh currents recorded at the same level of holding potential (−50 mV) in a Ringer solution containing 30 μM-curare. Records in *A* and *B* were obtained from two different neurones which exhibited type I slow e.p.s.c. (*A*) and type II e.p.s.c. (*B*). The period of application of ACh (100 μM) to the bath is indicated by arrows. *A* and *B*, top records were control muscarinic ACh currents produced by bath application of ACh (100 μM). Second traces were obtained in a 10 mM-K^+ Ringer solution. Third traces were obtained in Na^+-free choline Ringer solution. Bottom traces were obtained in the presence of 30 μM-curare and 10 μM-atropine.

Slow ACh currents in modified ionic media

The ionic dependency of the two types of muscarinic slow inward currents was analysed. Fig. 6 illustrates the effect of high-K^+ (10 mM) Ringer solution and Na^+-free solution on the slow ACh-induced currents recorded at −55 mV. Type I response (*A*) was markedly reduced in high-K^+ Ringer solution, while in Na^+-free choline Ringer solution, it was only slightly reduced in amplitude. On the other hand, type II

response (*B*) was markedly reduced by removal of extracellular Na^+. In high-K^+ Ringer solution, the type II response was only slightly depressed. Both type I and type II responses were identical to those of a slow e.p.s.c., because atropine (10 μM) eliminated both of them (Fig. 6).

Time course of slow e.p.s.c.

The duration of slow e.p.s.c.s was $37{\cdot}6 \pm 8{\cdot}7$ s ($n = 26$) with the falling phase (decay phase) lasting from 15 to 40 s. It was clear from the previous data that the time course of the falling phase of slow e.p.s.c.s depended on the membrane potential (cf.

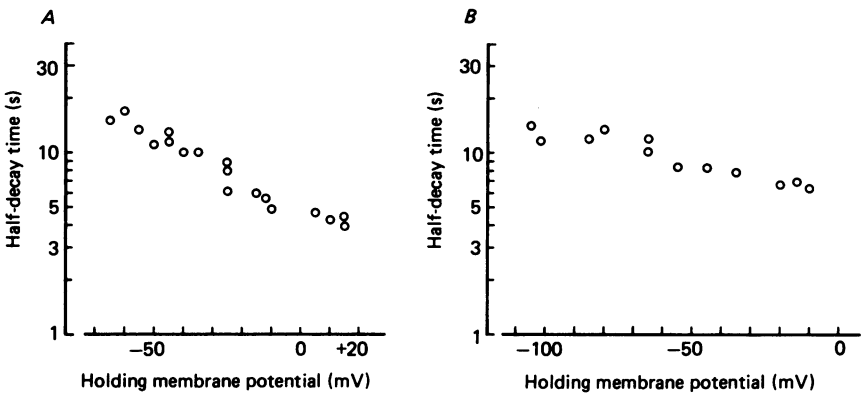

Fig. 7. The relationship between the half-decay time of type I slow e.p.s.c. (*A*) and type II slow e.p.s.c. (*B*) and the holding potential level. The half-decay times were obtained by measuring the time to half-amplitude from the peak of a slow e.p.s.c. Data of *A* and *B* were obtaind from records in Fig. 3 and Fig. 2, respectively. Ordinate: indicates the half-decay times on a log scale. Abscissa: indicates holding membrane potential.

Figs. 3–4). At depolarized potential levels, the slow e.p.s.c.s recovered quickly to the original current level after their peak amplitude. In contrast, the falling phase of slow e.p.s.c.s proceeded with a much slower time course at negative potential levels. Fig. 7 shows the relationship between holding membrane potentials and the half-decay time of a type I (*A*) and a type II (*B*) slow e.p.s.c. recorded at various holding potentials. As can be seen in this Figure, an exponential relationship was obtained: hyperpolarization of the ganglion cell's membrane potential increased the value of half-decay time, while depolarization markedly decreased the half-decay time. The potential dependency of half-decay time of type I slow e.p.s.c. was different from that of type II slow e.p.s.c. It appears that the decay phase of a type I slow e.p.s.c. is more dependent on membrane potential than that of type II, since the slope of type II potential dependency is less than that of type I.

DISCUSSION

The sympathetic ganglion cells examined in the present experiments clearly demonstrated at least two types of slow e.p.s.c. The type I response is associated with mainly a decrease in K^+ conductance, while the type II response is probably associated with an increased conductance to Na^+ and other cations.

The characteristics of the type I slow e.p.s.c. were quite similar to those observed by Kuba & Koketsu (1976: type 3 cell) and Adams & Brown (1982), i.e.: (i) membrane conductance was decreased during a type I slow e.p.s.c.; (ii) the amplitude of type I slow e.p.s.c. was increased at membrane potentials of -55 and -10 mV, was decreased at hyperpolarized potential levels, and at -60 to -70 mV was nullified and did not reappear even at more hyperpolarized levels; (iii) there was a suppression of the slow inward relaxation which reflects the closure of M-channels (Brown & Adams, 1980).

On the other hand, the characteristics of the type II slow e.p.s.c. were different from those of the type I response and rather similar to those of slow e.p.s.p.s reported by Nishi *et al.* (1969) and Kuba & Koketsu (1976: type 1 cell). Type II responses were: (i) characterized by a membrane conductance increase; (ii) increased in amplitude during a conditioning hyperpolarization (while Type I responses were increased during a conditioning depolarization) and nullified at $+5$ mV and (iii) primarily dependent on changes in external Na^+. These characteristics could not be explained by a reduction of K^+ conductance but rather by an increase of Na^+ or of Na^+ and some other cations, probably Ca^{2+} and K^+ (Kuba & Koketsu, 1976, 1978). It is of interest that the type II slow e.p.s.c. was nullified at levels more positive than $+5$ mV. This may suggest that the conductance system for Na^+ or other cations involved in Type II responses is also voltage dependent.

A mixed type of slow e.p.s.c. was also observed in about nine cells (about 20%) of the forty-three ganglion cells from which we recorded. The relationship between the amplitude of the slow e.p.s.c. and membrane potential appeared to be V-shaped (see Fig. 5); the bottom of this curve was -70 mV. In the present experiments, we did not analyse the ionic mechanism underlying such a mixed type of slow e.p.s.c., but it may be that the mixed-type slow e.p.s.c. is composed of both type I and type II responses. At depolarized potential levels, the characteristics of a type I slow e.p.s.c. were apparent while at hyperpolarized levels the type II response was unmasked due to the closing of M-channels. Such a mixed type of slow e.p.s.c. may correspond to the slow e.p.s.p. of type 2 cell reported by Kuba & Koketsu (1976, 1978). Thus, it seems that approximately one-third of slow e.p.s.c.s recorded in sympathetic neurones cannot be due solely to selective suppression of the M-current.

Adams & Brown (1982) reported that they observed on rare occasions an inward current at potentials positive to -70 mV in response to application of a muscarinic agonist. They concluded that this current did not contribute significantly to the normal slow e.p.s.p. since the normal resting potential of these neurones was between -50 and -55 mV. Mean resting potentials of -54 mV (Blackman, Ginsborg & Ray, 1963), -50 mV (Tosaka *et al.* 1968) and -65 mV (Koketsu & Yamada, 1982) have been reported for neurones impaled with a micro-electrode. Tosaka, Tasaka, Miyazaki & Libet (1983) have estimated that the resting potential of intact sympathetic neurones is at least equal to or negative to -65 mV. If such were the case, it would be expected that the M-current might not play a significant role in generation of the slow e.p.s.p. in intact sympathetic neurones.

A point of contention about the several investigations concerning the slow e.p.s.p. is whether or not there is a true reversal (for review see Kuba & Koketsu, 1978). Weight & Votava (1970) reported a clear reversal of the slow e.p.s.p. at the equilibrium potential for K (E_K). On the other hand, Kobayashi & Libet (1970)

observed only a partial reversal of the initial phase of the slow e.p.s.p. at membrane potentials more positive than the E_K similar to that recently reported for slow e.p.s.c.s by Kuffler & Sejnowski (1983) using a single-electrode voltage clamp. We, as well as Adams & Brown (1982), did not observe a reversal of the slow e.p.s.c. using the two-electrode voltage-clamp method. Similar results have been reported by the above investigators in a later study (Kobayashi & Libet, 1974) and by others (Nishi *et al.* 1969; Kuba & Koketsu, 1974, 1976). Libet (1971) suggested that the reversal of the slow e.p.s.p. was due to contamination by the slow i.p.s.p., particularly when nicotine was used to block the fast e.p.s.p. It is possible that some antagonists used to block nicotinic transmission in ganglia, or other drug treatments, affect membrane conductances and thereby either facilitate or prevent reversal of the slow e.p.s.p.

In the present experiments, we demonstrated that the decay phase of slow e.p.s.c.s of either type I or type II depended upon membrane potential. The half-decay time of slow e.p.s.c.s was increased and decreased by membrane hyperpolarization and depolarization, respectively. Such a voltage-dependent nature of the falling phase of slow e.p.s.c.s suggested that the falling phase of slow e.p.s.c. was not determined by the time for the removal of ACh from the receptor or synaptic cleft. The time course of the falling phase of slow e.p.s.c.s is too slow to be explained by either: (1) the activating and deactivating time constant of delayed rectification or (2) the M-channel (Brown & Adams, 1980). It is possible that the falling phase of the slow e.p.s.c. may be dependent on the formation of a second messenger.

The presence of a mixed type of response suggests that two types of muscarinic receptors exist on one neurone. Recently, Katayama & Nishi (1982) observed two types of peptidergic late slow e.p.s.c. in bull-frog sympathetic ganglion cells. The proportion of neurones having type I, type II, or the mixed type of muscarinic slow e.p.s.c.s in our experiments was different from that reported by Katayama & Nishi (1982). The discrepancy between the proportions suggests that the distinction between types is based on receptor distribution rather than a cell property. On the other hand, Kuffler & Sejnowski (1983) recently reported that muscarinic slow e.p.s.c.s and peptidergic late slow e.p.s.c.s recorded in the same neurone had the same voltage dependence and exhibited the same conductance changes. Their data suggest that the proportion of type I/type II/mixed type responses is linked to a property of the cell membrane rather than the receptor.

This work was supported by the Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan and by NS 16628.

REFERENCES

ADAMS, P. R. & BROWN, D. A. (1982). Synaptic inhibition of the M-current: slow excitatory post-synaptic potential mechanism in bullfrog sympathetic neurones *J. Physiol.* **332**, 263–272.

ADAMS, P. R., BROWN, D. A. & CONSTANTI, A. (1982). Pharmacological inhibition of the M-current. *J. Physiol.* **332**, 223–262.

AKASU, T. & KOKETSU, K. (1981). Voltage-clamp studies of a slow inward current in bullfrog sympathetic ganglion cell. *Neurosci. Lett.* **26**, 259–262.

BLACKMAN, J. G., GINSBORG, B. L. & RAY, C. (1963). Synaptic transmission in the sympathetic ganglion of the frog. *J. Physiol.* **167**, 353–373.

BROWN, D. A. & ADAMS, P. R. (1980). Muscarinic suppression of a novel voltage-sensitive K^+-current in a vertebrate neurone. *Nature, Lond.* **283**, 673–676.

KATAYAMA, Y. & NISHI, S (1982). Voltage-clamp analysis of peptidergic slow depolarizations in bullfrog sympathetic ganglion cells. *J. Physiol.* **333**, 305–313.

KOBAYASHI, H. & LIBET, B. (1970). Actions of noradrenaline and acetylcholine on sympathetic ganglion cells *J. Physiol.* **208**, 353–372.

KOBAYASHI, H. & LIBET, B. (1974). Is inactivation of potassium conductance involved in slow postsynaptic excitation of sympathetic ganglion cells? Effects of nicotine. *Life Sci. Oxford* **14**, 1871–1883.

KOKETSU, K. (1969). Cholinergic synaptic potentials and the underlying ionic mechanisms. *Fedn Proc.* **28**, 101–102.

KOKETSU, K. & YAMADA, M. (1982). Presynaptic muscarinic receptors inhibiting active acetylcholine release in the bullfrog sympathetic ganglion. *Br. J. Pharmac.* **77**, 75–82.

KUBA, K. & KOKETSU, K. (1974). Ionic mechanism of the slow excitatory postsynaptic potential in bullfrog sympathetic ganglion cells. *Brain Res.* **81**, 338–342.

KUBA, K. & KOKETSU, K. (1976). Analysis of the slow excitatory postsynaptic potential in bullfrog sympathetic ganglion cell. *Jap. J. Physiol.* **26**, 647–664.

KUBA, K. & KOKETSU, K. (1978). Synaptic events in sympathetic ganglia. *Prog. Neurobiol.* **11**, 77–169.

KUFFLER, S. W. & SEJNOWSKI, T. J. (1983). Peptidergic and muscarinic excitation at amphibian sympathetic synapses. *J. Physiol.* **341**, 257–278.

LIBET, B. (1971). Inactivation of potassium in slow post-synaptic excitation. *Science, N.Y.* **172**, 503–504.

LIBET, B., CHICHIBU, S. & TOSAKA, T. (1968). Slow synaptic responses and excitability in sympathetic ganglia of the frog. *J. Neurophysiol.* **31**, 383–395.

NISHI, S. & KOKETSU, K. (1960). Electrical properties and activities of single sympathetic neurons in frogs. *J. cell. comp. Physiol.* **55**, 15–30.

NISHI, S., SOEDA, H. & KOKETSU, K. (1965). Studies on sympathetic B and C neurones and patterns of preganglionic innervation. *J. cell. comp. Physiol.* **66**, 19–32.

NISHI, S., SOEDA, H. & KOKETSU, K. (1969). Unusual nature of ganglionic slow EPSP studied by a voltage-clamp method. *Life Sci. Oxford* **8**, 33–42.

SKOK, V. I. (1973). *Physiology of Autonomic Ganglia*, pp. 65–70. Tokyo: Igaku Shoin.

TOSAKA, T., CHICHIBU, S. & LIBET, B. (1968). Intracellular analysis of slow inhibitory and excitatory postsynaptic potentials in sympathetic ganglia of the frog. *J. Neurophysiol.* **31**, 396–409.

TOSAKA, T., TASAKA, J., MIYAZAKI, T. & LIBET, B. (1983). Hyperpolarization following activation of K^+ channels by excitatory postsynaptic potentials. *Nature, Lond.* **308**, 148–150.

WEIGHT, F. F. & VOTAVA, J. (1970). Slow synaptic excitation in sympathetic ganglion cells: evidence for synaptic inactivation of potassium conductance. *Science, N.Y.* **170**, 755–758.