Br. J. Pharmac. (1968), **34**, 177–188.

Effects of physostigmine on the afterdischarge and slow postsynaptic potentials of bullfrog sympathetic ganglia

K. KOKETSU, S. NISHI AND Y. NODA

Neurophysiology Laboratory, Department of Pharmacology and Therapeutics, Stritch School of Medicine, Loyola University, Hines, Illinois

1. The effects of anticholinesterases (anti-ChEs) (physostigmine, prostigmine and TEPP) on the afterdischarges and the extracellular and intracellular slow potentials of bullfrog sympathetic ganglia were studied.

2. The anti-ChEs augmented the early afterdischarge, the late negative potential and the slow excitatory postsynaptic potential. This indicated that the nature of the early afterdischarge was cholinergic (muscarinic) and that the late negative potential or the slow excitatory postsynaptic potential generated the early afterdischarge.

3. Since the anti-ChEs increased the positive potential, the depression of the early afterdischarge observed in the presence of an antiChE was explained to be caused by the increased inhibitory effect of the enhanced positive potential.

4. Prostigmine and tetraethyl pyrophosphate did not show any appreciable effects on the late afterdischarge, the late late negative potential nor the late slow excitatory postsynaptic potential. This indicated that the nature of the late afterdischarge was non-cholinergic and that the late late negative potential or the late slow excitatory postsynaptic potential generated the late afterdischarge.

5. Physostigmine reversibly depressed the late afterdischarge, the late late negative potential and the late slow excitatory postsynaptic potential. The depressant action of physostigmine was not due to its anti-ChE action.

It has long been known that repetitive stimulation of the preganglionic nerve fibres of mammalian sympathetic ganglia is followed by a long-lasting afterdischarge (AD) of the postganglionic nerve fibres (Bronk, 1939 ; Eccles, 1944 ; Larrabee & Bronk, 1947), which is enhanced in the presence of anticholinesterases (anti-ChEs) and blocked by atropine or procaine but not by (+)-tubocurarine ((+)-TC) (Emmelin & MacIntosh, 1956 ; Takeshige & Volle, 1962, 1963 ; Volle, 1962). More recently, the AD of amphibian sympathetic ganglia has been described to be composed of two distinctly different components ; the early afterdischarge (EAD) and the late afterdischarge (LAD), which are, respectively, sensitive and insensitive to blockade by atropine (Nishi & Koketsu, 1966, 1968a). Because both the EAD and LAD are enhanced in the presence of nicotine or (+)-TC, it has been suggested that the muscarinic and certain non-cholinergic receptors are responsible for the production of the EAD and LAD, respectively (Nishi & Koketsu, 1966, 1968a).

In the curarized superior cervical ganglion of both turtles and rabbits, preganglionic volleys evoke a sequence of slow potentials (Eccles, 1952; Laporte & Lorente de No, 1950), which are composed of the P (positive) and the LN (late negative) potentials (Eccles & Libet, 1961; Libet, 1962a, b). The blocking actions of botulinium toxin and atropine on the LN potential as well as the P potential of mammalian sympathetic ganglia indicate that cholinergic transmission is involved in the mediation of both the LN and P potentials (Eccles & Libet, 1961). The slow IPSP (inhibitory postsynaptic potential) and the slow EPSP (excitatory postsynaptic potential) which correspond to the P and the LN potential, respectively, were recorded intracellularly from the postganglionic neurone of rabbits (Libet & Tosaka, 1966; Tosaka & Libet, 1965).

In the case of amphibian lumbar sympathetic ganglia, in addition to the slow IPSP and EPSP (Libet & Tosaka, 1966; Nishi & Koketsu, 1968a; Tosaka & Libet, 1965), the late slow EPSP which corresponds to the LLN (late late negative) potential has recently been recorded (Nishi & Koketsu, 1966, 1968a). Inasmuch as the time course and pharmacological properties of the slow EPSP and the late slow EPSP were very similar to those of the EAD and LAD, respectively, it was suggested that the slow EPSP (LN) and the late slow EPSP (LLN) potentials were responsible for the production of the EAD and LAD, respectively (Nishi & Koketsu, 1966, 1968a).

It has been reported that the P potential is enhanced while the LN potential is depressed in the presence of an anti-ChE (Eccles, 1952). Libet (1967), however, presented experimental evidence that the LN potential could also be enhanced directly by the action of anti-ChEs. In the work to be reported, the effects of physostigmine and other anti-ChEs on the EAD and the LAD as well as on the slow potentials recorded extracellularly (P, LN and LLN potentials) or intracellularly (slow EPSP and late slow EPSP) were studied. The purpose of this study was to see whether these potential changes were cholinergic or non-cholinergic in nature, and to study the inter-relation between the ADs and the slow potentials.

Methods

Isolated paravertebral sympathetic chains of bullfrog (*Rana catesbeiana*) were used throughout. The preparation was perfused continuously with Ringer's solution (NaCl, 112 mM; $CaCl_2$, 1.8 mM; KCl, 2 mM; $NaHCO_3$, 2 mM) to which a desired amount of a drug was added to test its effect. The experimental procedure for recording the ADs of the postganglionic nerve fibres, the extracellular slow potentials and the intracellular slow potentials of the postganglionic neurones has been fully described elsewhere (Nishi & Koketsu, 1968a, b). The concentrations of drugs used were as follows, unless stated otherwise: physostigmine sulphate $1–5 \times 10^{-5}$M; prostigmine methylsulphate $1–5 \times 10^{-5}$M; tetraethyl pyrophosphate (TEPP), $1–5 \times 10^{-6}$M; atropine sulphate, 2×10^{-5}M; nicotine sulphate, 1×10^{-4}M. All experiments were carried out at room temperature (22°–23° C).

Results

Afterdischarge

Effect on the EAD

In bullfrog sympathetic ganglia, the EAD has been defined as the AD which is sensitive to blockade by atropine (Nishi & Koketsu, 1966, 1968a). The AD produced

in response to tetanic stimulation of the preganglionic B and C fibres (Nishi, Soeda & Koketsu, 1965) are composed of the EAD as well as the LAD, whereas that produced in response to tetanic stimulation of the preganglionic B fibres alone consists only of the EAD. In the following experiments, therefore, the effects of physostigmine and other anti-ChEs on the EAD were studied by recording the AD produced by stimulating the preganglionic B fibres alone.

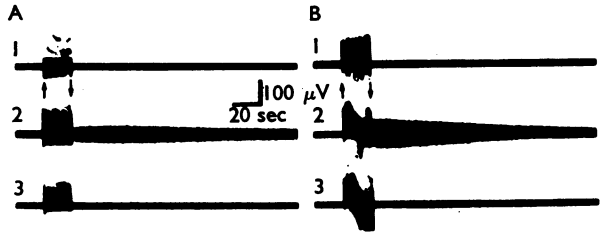
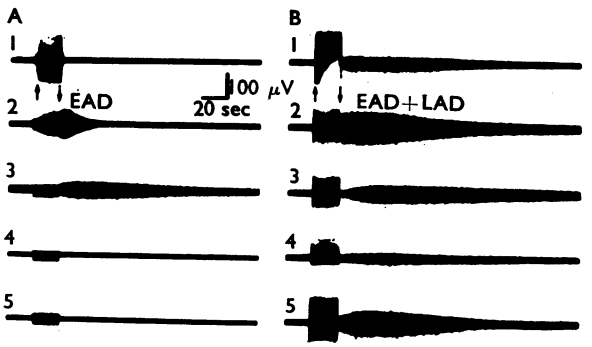


FIG. 1. AD produced in the presence of TEPP; the ADs produced in response to tetanic stimulation (10/sec for 20 sec) of the preganglionic B and B plus C fibres are shown in records A (1–3) and records B (1–3), respectively. A-1, B-1: In Ringer's solution; no ADs are seen. A-2, B-2: 10 min after application of TEPP (1×10^{-6}M); marked ADs are seen in both records. Atropine (2×10^{-5}M) was then added. A-3, B-3: 20 min after addition of atropine; the ADs are almost completely abolished. Arrows indicate beginning and cessation of stimulation.

FIG. 2. Effect of physostigmine on the AD of a nicotinized preparation; the ADs produced in response to tetanic stimulation (10/sec for 20 sec) of the preganglionic B and B plus C fibres are shown in records A (1–5) and records B (1–5), respectively. A-1, B-1: In Ringer solution; the AD is seen only in B-1. A-2, B-2: 40 min after application of nicotine (1×10^{-4}M); the short-lived (EAD) and long-lasting AD (EAD and LAD) are seen in A-2 and B-2, respectively. Physostigmine (5×10^{-5}M) was then added. A-3, B-3: 20 min after addition of physostigmine; the ADs produced during and immediately after stimulation are depressed in both records, while a slight prolongation of the AD is seen in B-3. Atropine (2×10^{-5}M) was then added. A-4, B-4: 20 min after addition of atropine; the AD is abolished in A-4; while it is strongly depressed in B-4. Physostigmine was then withdrawn, thus the solution contained nicotine and atropine. A-5, B-5: 20 min after withdrawal of physostigmine; no AD is seen in A-5; while a marked restoration of the AD is seen in B-5. Arrows indicate beginning and cessation of stimulation.



Normal ganglia. In Ringer's solution, tetanic stimulation (10/sec for 20 sec) of the preganglionic B fibres initiated no detectable EAD (A-1 of Figs. 1 and 2). Following addition of physostigmine (5×10^{-5}M), a marked AD which lasted for about 1 min was produced in response to the same preganglionic stimulation. The effect of prostigmine in the same concentration was similar to that of physostigmine. A stronger effect was observed with TEPP, an AD lasting for a few minutes being produced (A-2 of Fig. 1). The AD produced in the presence of an anti-ChE was abolished within 10–20 min following addition of atropine (A-3 of Fig. 1).

Nicotinized ganglia. When the excitatory postsynaptic potential (EPSP) was completely blocked by nicotine, tetanic stimulation (10/sec for 20 sec) of the preganglionic B fibres invariably evoked a marked but short-lived AD, namely, the EAD (A-2 of Fig. 2). Adding physostigmine to the solution always depressed the AD but prolonged its duration, as seen in record A-3 of Fig. 2. A similar effect was observed with prostigmine or TEPP. The AD produced in the presence of an anti-ChE was abolished by atropine (A-4 of Fig. 2).

Effect on the LAD

The LAD of the bullfrog sympathetic ganglia has been defined as the AD which is insensitive to blockade by atropine, and can be produced only when the preganglionic C fibres are excited (Nishi & Koketsu, 1966, 1968a). When stimulation was applied to the preganglionic B and C fibres of the normal or nicotinized ganglia, an AD composed of both the EAD and the LAD was produced. The EAD, however, could be abolished by applying atropine to the preparation.

Normal ganglia. In most preparations, tetanic stimulation (10/sec for 20 sec) of the preganglionic B and C fibres initiated no AD in Ringer solution. These preparations produced a marked AD in response to stimulation following application of an anti-ChE, such as physostigmine, prostigmine or TEPP (B-2 of Fig. 1). The AD thus produced was abolished by atropine (B-3 of Fig. 1). In some preparations, similar tetanic stimulation of the preganglionic B and C fibres evoked an AD, which consisted of the EAD and LAD, in Ringer solution. The AD of these preparations was markedly enhanced in the presence of an anti-ChE. The enhanced AD was almost but not completely abolished by atropine ; the AD which remains in the presence of atropine would be the LAD. When atropine was applied to these preparations before addition of an anti-ChE, the EAD disappeared, whereas the LAD remained unaffected (Nishi & Koketsu, 1966, 1968a). Subsequent application of physostigmine to the preparation markedly depressed the LAD, whereas that of prostigmine or TEPP did not affect it. These observations were confirmed in the following experiments.

Nicotinized ganglia. With preparations treated with nicotine for more than 30 min, tetanic stimulation (10/sec for 20 sec) of the preganglionic B and C fibres always initiated an AD composed of the EAD and the LAD (B-2 of Fig. 2).

Application of physostigmine to the nicotinized preparation invariably resulted in a depression of the AD, particularly of its initial part (B-3 of Fig. 2) ; the duration of the AD, however, was prolonged. Similar effects were observed with prostigmine and TEPP. When atropine was added, the AD was strongly depressed (B-4 of Fig. 2). The AD, however, was found to be markedly restored (B-5 of Fig. 2), if physostigmine alone was withdrawn. This would indicate that physostigmine

depressed the LAD, inasmuch as the AD initiated in the presence of atropine and physostigmine would also consist only of the LAD. Such a restoration of the AD was not observed when prostigmine was used. The depressant action of physostigmine on the LAD was further confirmed in the following experiment.

As records 1 and 2 of Fig. 3 show, on application of atropine to the nicotinized preparation, the initial part of the AD (EAD) was abolished, whereas the late part (LAD) was unaffected or occasionally augmented. Addition of prostigmine or TEPP to the solution had no appreciable effect on the LAD (3 of Fig. 3). Addition of physostigmine, however, always resulted in a strong depression of the LAD (4 and 5 of Fig. 3); the depressant action of physostigmine on the LAD was partially reversible (6 of Fig. 3).

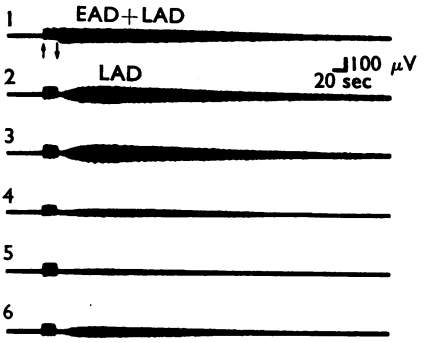


FIG. 3. Effects of TEPP and physostigmine on the AD produced in the presence of nicotine and atropine in response to tetanic stimulation of the preganglionic B and C fibres. 1: AD (EAD and LAD) recorded 50 min after application of nicotine (1×10^{-4}M). Atropine (2×10^{-5}M) was then added. 2: AD (LAD) recorded 20 min after application of atropine. TEPP (5×10^{-6}M) was added after this record was taken. 3: 60 min after application of TEPP; no appreciable changes in the AD are seen. Physostigmine (5×10^{-5}M) was then added. 4 and 5: 30 and 50 min, respectively, after application of physostigmine. Physostigmine was then withdrawn, thus the solution contained nicotine, atropine and TEPP. 6: 20 min after withdrawal of physostigmine; a restoration of the AD is seen. Arrows indicate beginning and cessation of stimulation.

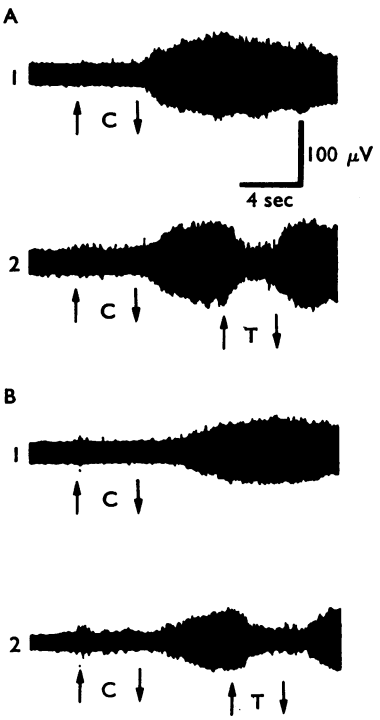


FIG. 4. Effects of physostigmine on the inhibition of the AD of a nicotinized preparation; tetanic stimulation of a short duration (10/sec for approximately 4 sec) of the preganglionic B and C fibres were used for both conditioning (marked by C) and test (marked by T) stimulations. Records A and B were taken before and after application of physostigmine (1×10^{-5}M), respectively; the ADs produced by conditioning stimulation and test stimulation are shown in 1 and 2, respectively. Arrows indicate beginning and cessation of stimulation.

Effect of inhibition of AD

In the nicotinized preparations, inhibition of the AD can be observed if tetanic test stimulation is applied either to the preganglionic B or B and C fibres during the development of the AD. As seen in record A-2 of Fig. 4, inhibition of the AD occurs during and immediately after cessation of test stimulation and is apparently caused by the P potential produced in response to test stimulation.

Nicotinized ganglia. Tetanic stimulation (10/sec for approximately 4 sec) was applied to the preganglionic B and C fibres to produce the AD (conditioning stimulation), and then to see its inhibitory effect on the AD (test stimulation). When conditioning stimulation was applied for such a short duration, the AD, instead of being produced during stimulation, was initiated immediately after stimulation was discontinued (A-1 of Fig. 4). If test stimulation was subsequently applied during the development of the AD, the AD showed a marked depression (A-2 of Fig. 4). The initiation of the AD in response to conditioning stimulation was markedly delayed in the presence of physostigmine, and the overall discharges were significantly reduced (B-1 of Fig. 4). In this case, a much stronger inhibition that that observed in the presence of physostigmine was seen on application of test stimulation (B-2 of Fig. 4). Similar results were obtained with prostigmine and TEPP. The pronounced inhibition of the AD may be due to the enhancement of the P potential.

Nicotinized ganglia treated with ouabain. It has been reported that the P potential is selectively depressed, and the inhibition of the AD disappears when ouabain (1×10^{-5} to 1×10^{-6}M) is applied to the nicotinized preparations (Nishi & Koketsu, 1967). In the presence of ouabain in this concentration, application of an anti-ChE greatly augmented the AD produced by stimulating either the preganglionic B or B and C fibres (Fig. 5). This can be compared with the observation that anti-ChEs have a depressant effect on the AD of nicotinized preparations. It may be that the P potential, suppressed by ouabain, cannot be augmented sufficiently to overwhelm the concomitant enhancement of the LN potential, which is actually the generator potential of the EAD.

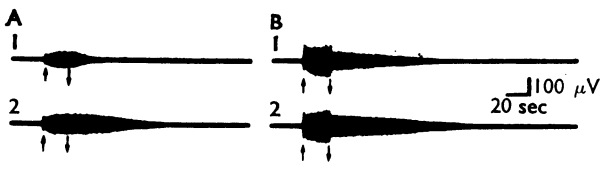


FIG. 5. Effect of physostigmine on the AD of a nicotinized preparation in the presence of ouabain (1×10^{-5}M); the ADs produced in response to tetanic stimulation (10/sec for 20 sec) of the preganglionic B and B plus C fibres are shown in records A (1–2) and records B (1–2), respectively. 1 and 2 in these records were taken before and 20 min after application of physostigmine (1×10^{-5}M). Note the marked enhancement of the ADs in the presence of physostigmine. Arrows indicate beginning and cessation of stimulation.

Extracellular slow potential

Effect on the LN potential

The LN potential of the bullfrog sympathetic ganglia has been defined as the slow negative potential recorded extracellularly by means of the sucrose-gap method, and as being sensitive to atropine but insensitive to nicotine or (+)-TC. The slow negative potential produced in response to stimulation of the preganglionic B and C fibres is composed of the LN as well as the LLN potential, whereas that produced by stimulating the preganglionic B fibres alone consists only of the LN potential (Nishi & Koketsu, 1966, 1968a). Accordingly, the effects of physostigmine and other anti-ChEs on the LN potential were studied by recording the slow negative potential produced by stimulating the preganglionic B fibres.

Normal ganglia. In Ringer solution, when tetanic stimulation (10/sec for 4 sec) was applied to the preganglionic B fibres, a slow positive potential was initiated immediately upon cessation of stimulation, and was followed by a very slow negative potential (1 of Fig. 6). The initial slow positive potential consisted of the after-hyperpolarization of the action potential, and probably also the P potential of ganglion cells. That the following slow negative potential was sensitive to atropine indicated that this was the LN potential (1 of Fig. 6); this potential was abolished, whereas the initial positive potential simply returned to the original potential level in the presence of atropine. If physostigmine or prostigmine (1×10^{-5}M) was added to Ringer solution, the initial positive slow potential disappeared and a marked slow negative potential, which developed during stimulation and lasted for more than a few minutes, was initiated in response to the same preganglionic stimulation (2 of Fig. 6). A similar effect was observed with TEPP in a concentration of 1×10^{-6}M. The enhanced slow negative potential was depressed and shortened within 10–20 min following addition of atropine (3 of Fig. 6), while its initial part, particularly that which developed during stimulation, was not appreciably affected; this part of the potential was abolished by nicotine, indicating that it is the so-called N (negative) potential (compare Eccles & Libet, 1961; Libet, 1962a, b) which corresponds to the EPSP. It should be mentioned here that a marked EAD was initiated under the experimental condition where record 2 of Fig. 6 was obtained, and was abolished under the experimental condition where record 3 of Fig. 6 was obtained.

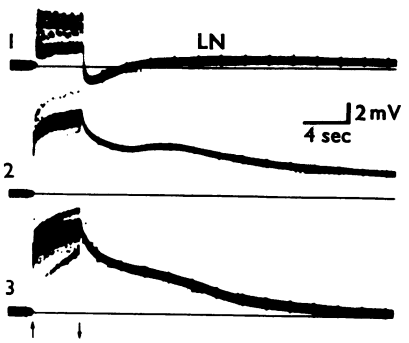


FIG. 6. Effect of physostigmine on the extracellular slow negative potential produced in response to tetanic stimulation (10/sec for 4 sec) of the preganglionic B fibres. 1: In Ringer solution; note the LN potential. 2: 20 min after application of physostigmine (1×10^{-5}M); a marked slow negative potential developed during and after stimulation. Atropine (2×10^{-5}M) was then added. 3: 20 min after application of atropine; note a marked depression of the slow negative potential (see text for details). Arrows indicate beginning and cessation of stimulation.

Nicotinized ganglia. Preganglionic B fibre stimulation of the nicotinized preparations evoked a P potential which was followed by a LN potential (A-1 of Fig. 8); both the LN and P potentials were abolished by addition of atropine. When an anti-ChE, such as physostigmine, prostigmine or TEPP, was applied to the nicotinized preparations, the LN potential either was depressed (Eccles, 1952) or remained almost unchanged (A-2 of Fig. 8), whereas the P potential was markedly enhanced. The absence of augmentation of the LN potential is probably due to the concomitant and even greater augmentation of the P potential (compare Libet, 1967). It should be mentioned here that the EAD was depressed under the same experimental circumstances.

Effect on the LLN potential

The LLN potential of the bullfrog sympathetic ganglia has been defined as the slow negative potential which is insensitive to blockade by atropine, and can be produced only when the preganglionic C fibres are excited (Nishi & Koketsu, 1966, 1968a). When stimulation was applied to the preganglionic B and C fibres of the normal or nicotinized ganglia, the slow negative potential composed of the LN and LLN potentials was initiated. The LN potential, however, could be abolished with atropine. The effects of anti-ChEs on the LLN potentials of the nicotinized ganglia treated with atropine were studied in the following experiments.

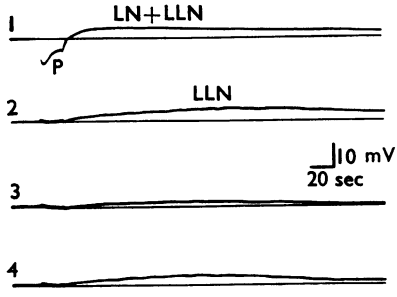


FIG. 7. Effect of physostigmine on the extracellular slow negative potential of a nicotinized preparation produced in response to tetanic stimulation (10/sec for 20 sec) of the preganglionic B and C fibres, in the presence of atropine. 1: 40 min after application of nicotine (1×10^{-4}M); note the P, LN and LLN potentials. Atropine (2×10^{-5}M) was then added. 2: 20 min after application of atropine; note that the LLN potential remains. Physostigmine (5×10^{-5}M) was added after this record was taken. 3; 20 min after application of physostigmine; note the depression of the slow negative potential. Physostigmine was then withdrawn, thus the solution contained nicotine and atropine. 4: 30 min after withdrawal of physostigmine; note the partial restoration of the slow negative potential. Arrows indicate beginning and cessation of stimulation.

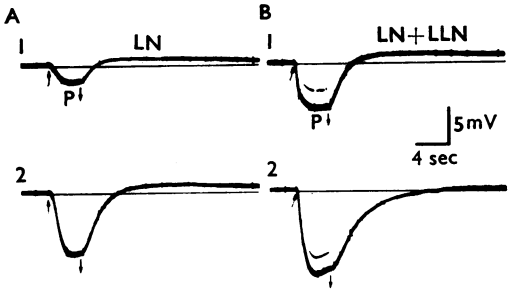


FIG. 8. Effect of physostigmine on the extracellular slow positive potential of a nicotinized preparation; the potentials produced in response to tetanic stimulation (10/sec for 4 sec) of the preganglionic B and B plus C fibres are shown in records A and B, respectively. A-1, B-1: 60 min after application of nicotine (1×10^{-4}M) and atropine (2×10^{-5}M); note the P as well as the LN and LLN potentials. Physostigmine (1×10^{-5}) was then added. A-2, B-2: 20 min after application of physostigmine; note that the slow positive potential is markedly enhanced, while the slow negative potentials are either unaffected or depressed. Arrows indicate beginning and cessation of stimulation.

Nicotinized ganglia. As seen in 1 of Fig. 7, the nicotinized ganglia produced a slow positive potential (P potential), which was followed by a long-lasting slow negative potential composed of the LN and LLN potentials, in response to tetanic stimulation (10/sec for 20 sec) of the preganglionic B and C fibres. Upon application of atropine, the P potential disappeared, but the slow negative potential consisting of the LLN potential remained (2 of Fig. 7). In such cases, addition of physostigmine markedly depressed the LLN potential (3 of Fig. 7); application of prostigmine or TEPP, on the other hand, showed no appreciable effect on the LLN potential. The depressant effect of physostigmine on the LLN potential was partially reversible (4 of Fig. 7).

Effect on the P potential

The P potential can be recorded when tetanic stimulation is applied either to the preganglionic B or B and C fibres of the nicotinized ganglia (Koketsu & Nishi, 1967; Nishi & Koketsu, 1967, 1968b).

Nicotinized ganglia. For this experiment relatively short tetanic stimulations (10/sec for 4 sec) were used (A-1 and B-1 of Fig. 8). A marked enhancement of the P potential was always seen when physostigmine was applied to the preparations (A-2 and B-2 of Fig. 8). Similar effects were observed with prostigmine and TEPP. The effects of physostigmine and prostigmine were reversible.

Intracellular slow potential

Effect on the slow EPSP

The slow EPSP of the bullfrog sympathetic ganglia has been defined as the intracellular slow depolarizing potential recorded from the postganglionic neurones in response to preganglionic stimulation, and as being sensitive to atropine but insensitive to nicotine or (+)-TC (Nishi & Koketsu, 1966, 1968a). When the preganglionic B fibres alone are stimulated, the intracellular slow depolarizing potential recorded from the postganglionic B or C cells (Nishi, Soeda & Koketsu, 1965) of a nicotinized ganglion consists only of the slow EPSP. When tetanic stimulation (10–50/sec) was applied to the preganglionic B (or B and C) fibres, the majority of B and C cells produced no visible intracellular hyperpolarization, viz., the slow IPSP (see, however, Libet & Tosaka, 1966; Tosaka & Libet, 1965). It was therefore tentatively assumed that the receptors responsible for the slow IPSP may be located on the axon membrane near the cell-body (Nishi & Koketsu, 1966, 1968a). The latency of the slow EPSP, which was measured as the time lag between the onset of tetanic stimulation and the initiation of the slow EPSP, was less than 1 sec (100–500 msec) (compare Libet, 1967).

The slow EPSP initiated by tetanic stimulation of the preganglionic B fibres is shown in record 1 of Fig. 9. If physostigmine was applied to the ganglion while the electrode impaled the cell, a marked enhancement of the slow depolarizing potential was observed within 5–10 min (2 of Fig. 9). With the addition of atropine, the enhanced slow depolarizing potential was abolished within 5 min (3 of Fig. 9). Similarly, a distinct effect of physostigmine on the slow EPSP was observed.

Effect on the late slow EPSP

The late slow EPSP of the bullfrog sympathetic ganglia has been defined as the intracellular slow depolarizing potential recorded from the postganglionic neurones in response to preganglionic stimulation, and as being insensitive to both atropine and nicotine or (+)-TC. When tetanic stimulation (10/sec for 20 sec) was applied to the preganglionic B and C fibres of the nicotinized ganglia, the slow intracellular depolarization recorded from the postganglionic B or C cells (Nishi, Soeda & Koketsu, 1965) was composed of the late slow EPSP and the slow EPSP (Nishi & Koketsu, 1966, 1968a). When atropine was applied to the preparations, the slow EPSP was abolished, whereas the late slow EPSP was unaffected. The latency of the late slow EPSP, which was measured as the time lag between the onset of tetanic stimulation and initiation of the late slow EPSP, was much longer than that of the slow EPSP.

The late slow EPSP initiated by tetanic stimulation of the preganglionic B and C fibres is shown in record 1 of Fig. 10. If physostigmine was applied to the ganglion while the electrode impaled the cell, a depression of the late slow EPSP was observed within 5 min (2 of Fig. 10); the depressant effect of physostigmine on the LLN potential was partially reversible (3 of Fig. 10). No significant changes in the late slow EPSP were observed when prostigmine was used.

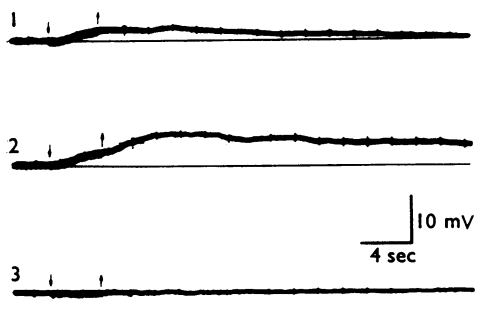
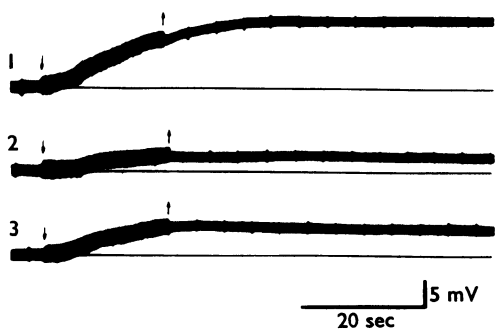


FIG. 9. Effect of physostigmine on the slow EPSP, recorded intracellularly from a single cell of a nicotinized preparation, produced in response to tetanic stimulation (10/sec for 4 sec) of the preganglionic B fibre. A cell was impaled with a microelectrode approximately 50 min after application of nicotine (record 1). Records 2 and 3 were obtained successively from the same cell 10 min after application of physostigmine (1×10^{-5}M) and 5 min after application of atropine (2×10^{-5}M), respectively. Arrows indicate beginning and cessation of stimulation.

FIG. 10. Effect of physostigmine on the late slow EPSP, recorded intracellularly from a single cell of a nicotinized preparation, produced in response to tetanic stimulation (10/sec for 20 sec) of the preganglionic B and C fibres, in the presence of atropine. A single cell was impaled with a microelectrode approximately 60 min after application of nicotine (1×10^{-4}M) and atropine (2×10^{-5}M) (record 1). Record 2 was obtained from the same cell 10 min after application of physostigmine (1×10^{-5}M), and record 3 was obtained 10 min after withdrawal of physostigmine. Arrows indicate beginning and cessation of stimulation.



Discussion

The AD produced in Ringer solution in response to stimulation of the preganglionic B fibres was enhanced in the presence of an anti-ChE. It is clear that the enhanced AD is a component of the EAD, because it is abolished by atropine. The fact that the EAD of the nicotinized preparation was depressed in the presence of an anti-ChE could be due to the enhancement of the P potential. Indeed, the very pronounced inhibition of the AD was demonstrated with nicotinized preparations in the presence of an anti-ChE. Furthermore, in the case of nicotinized preparations treated with ouabain which depressed the P potential the EAD was markedly increased in the presence of an anti-ChE. The reason why the EAD of normal (non-nicotinized) preparations was enhanced in the presence of an anti-ChE was because the N potential (EPSP), which was increased and prolonged, antagonized the development of the P potential, and consequently obscured the inhibitory effect of the P potential. In such circumstances, the LN potential (slow EPSP), which was actually enhanced in the presence of an anti-ChE, would augment the EAD. It is clear that the N potential does not generate the afterdischarges, because they are abolished by atropine despite the fact that the N potential is markedly increased and prolonged.

The extracellular slow negative potential produced in response to stimulation of the preganglionic B fibres was markedly increased and prolonged in Ringer solution containing an anti-ChE. That the late part of the enhanced slow negative potential was depressed by atropine suggested that the LN potential was augmented in the presence of an anti-ChE. The fact that the LN potential of the nicotinized preparations was not enhanced with an anti-ChE was clearly due to the concomitant and predominant increase of the P potential. The intracellular slow depolarization recorded from a nicotinized preparation by stimulating the preganglionic B fibres was markedly increased and prolonged in the presence of an anti-ChE. The enhanced slow depolarization was completely abolished by atropine, indicating that the slow EPSP was definitely enhanced by an anti-ChE. These results indicated that both the LN potential and the slow EPSP were cholinergic and were the generator potentials of the EAD, as suggested in an earlier work (Nishi & Koketsu, 1966, 1968a).

The AD produced in Ringer solution by stimulating the preganglionic B and C fibres was enhanced by an antiChE, but the enhanced AD was almost completely abolished by atropine, showing no sign that the component of the LAD had been enhanced. That the LAD produced from the nicotinized preparations treated with atropine was not affected by prostigmine or TEPP but was depressed by physostigmine indicates that the LAD is noncholinergic. The LLN potential was reversibly depressed by physostigmine but was not affected by prostigmine or TEPP. Similar results were observed with the late slow EPSP. These results indicated that both the LLN potential and the late slow EPSP were non-cholinergic, and were the generator potentials of the LAD, as suggested in previous works (Nishi & Koketsu, 1966, 1968a).

The mechanism underlying the depressant action of physostigmine on the late slow EPSP remains to be clarified. Because neither prostigmine nor TEPP exhibited such an action, it is probably not due to its anti-ChE action. Although the transmitter responsible for the production of the late slow EPSP is unknown, as noted

previously (Nishi & Koketsu, 1966, 1968a), it is interesting that physostigmine is, so far, the only agent found to block the LAD effectively.

This work was supported by National Science Foundation grant GB-5269X, and the Institute of Neurological Diseases and Blindness, NIH, Grant NB06672-02.

REFERENCES

BRONK, D. W. (1939). Synaptic mechanisms in sympathetic ganglia. *J. Neurophysiol.*, **2**, 380–401.

ECCLES, J. C. (1944). The nature of synaptic transmission in a sympathetic ganglion. *J. Physiol., Lond.*, **103**, 27–54.

ECCLES, R. M. (1952). Responses of isolated curarized sympathetic ganglia. *J. Physiol., Lond.*, **117**, 196–217.

ECCLES, R. M. & LIBET, B. (1961). Origin and blockade of the synaptic responses of curarized sympathetic ganglia. *J. Physiol., Lond.*, **157**, 484–503.

EMMELIN, N. G. & MACINTOSH, F. C. (1956). The release of acetylcholine from perfused sympathetic ganglia and skeletal muscles. *J. Physiol., Lond.*, **131**, 477–496.

KOKETSU, K. & NISHI, S. (1967). Characteristics of the slow inhibitory postsynaptic potential of bullfrog sympathetic ganglion cells. *Life Sci.*, **6**, 1827–1836.

LAPORTE, Y. & LORENTE DE NO, R. (1950). Potential changes evoked in a curarized sympathetic ganglion by presynaptic volleys of impulses. *J. cell comp. Physiol.*, **35**, Suppl. 2, 61–106.

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

LIBET, B. (1962a). Slow synaptic responses in sympathetic ganglia. *Fedn Proc.*, **21**, 345.

LIBET, B. (1962b). Slow excitatory and inhibitory synaptic responses in sympathetic ganglia. *Proc. XXII Int. Congr. Physiol. Sci.*, **2**, 809.

LIBET, B. (1967). Long latent periods and further analysis of slow synaptic responses in sympathetic ganglia. *J. Neurophysiol.*, **30**, 494–514.

LIBET, B. & TOSAKA, T. (1966). Slow postsynaptic potentials recorded intracellularly in sympathetic ganglia. *Fedn Proc.*, **25**, 455.

NISHI, S. & KOKETSU, K. (1966). Late after-discharges of sympathetic postganglionic fibers. *Life Sci.*, **5**, 1991–1997.

NISHI, S. & KOKETSU, K. (1967). Origin of ganglionic inhibitory postsynaptic potential. *Life Sci.*, **6**, 2049–2055.

NISHI, S. & KOKETSU, K. (1968a). The early and late after discharges of amphibian sympathetic ganglion cells. *J. Neurophysiol.*, **31**, 109–121.

NISHI, S. & KOKETSU, K. (1968b). An analysis of the slow inhibitory postsynaptic potential of bullfrog sympathetic ganglion. *J. Neurophysiol.*, in the Press.

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

TAKESHIGE, C. & VOLLE, R. L. (1962). Bimodal responses of sympathetic ganglia to acetylcholine following eserine or repetitive preganglionic stimulation. *J. Pharmac. exp. Ther.*, **138**, 66–73.

TAKESHIGE, C. & VOLLE, R. L. (1963). Asynchronous post-ganglionic firing from the cat superior cervical sympathetic ganglia treated with neostigmine. *Br. J. Pharmac. Chemother.*, **20**, 214–220.

TOSAKA, T. & LIBET, B. (1965). Slow postsynaptic potentials recorded intracellularly in sympathetic ganglia of frog. *Int. Congr. Physiol. Sci., 23rd, Tokyo, Japan.*

VOLLE, R. L. (1962). The actions of several ganglion-blocking agents on the postganglionic discharge induced by di*iso*propyl phosphorofluoridate (DFP) in sympathetic ganglia. *J. Pharmac. exp. Ther.*, **135**, 45–53.

(*Received May* 27, 1968)