

THE EFFECTS OF SUBSTANCE P AND BACLOFEN ON MOTONEURONES OF ISOLATED SPINAL CORD OF THE NEWBORN RAT

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SUMMARY

The effects of substance P (SP) and baclofen were studied in the isolated spinal cord of newborn rats. Potential changes generated in motoneurons were recorded extracellularly from the ventral root (L₃-L₅). When SP (8×10^{-8} M) was introduced into the bath, the depolarization of motoneurons began with a delay of 1.1 s. A large part of this delay can be explained as a time needed for SP to reach the site of action on spinal neurones.

When the preparation was perfused with artificial cerebrospinal fluid (CSF) containing low Ca (0.1 mM) and high Mg (1.6-3.5 mM), the spinal reflexes induced by dorsal root stimulation and recorded from the corresponding ventral root were completely abolished. The depolarizing action of SP (10^{-7} M) on the motoneurons was potentiated in the low-Ca medium, suggesting that SP acts directly on the motoneurons.

Baclofen at 10^{-6} M depressed the monosynaptic reflex by about 75%. The SP-induced depolarization of motoneurons was greatly depressed by baclofen in both normal and 0.1 mM-Ca mediums. The effects of baclofen (10^{-6} M) on the responses to various depolarizing agents were compared with that on the response to SP in artificial CSF containing 0.1 mM-Ca and 1.6-2 mM-Mg. The SP response was reduced by about 80%, whereas the responses to acetylcholine and glycine were not appreciably affected, and those to L-glutamate, GABA and noradrenaline were depressed by 10-22% by baclofen. These results suggest that baclofen blocks transmission at certain primary afferent synapses by antagonizing the action of SP that is released as a transmitter.

INTRODUCTION

Although the hypothesis that substance P (SP) is an excitatory transmitter of certain primary afferent neurones is supported by abundant neurochemical and immunohistochemical evidence (Cuello *et al.* 1978; Hökfelt *et al.* 1975; Otsuka & Takahashi, 1977), relatively little is known about the mode of action of SP at central synapses. As yet, SP-mediated synaptic potentials have not been demonstrated. It is therefore of crucial importance to study the action of SP at the synapses where its transmitter role is anticipated. The isolated spinal cord of the newborn rat (Otsuka & Konishi, 1974) provides an excellent opportunity for studying the action of SP on central neurones, because the peptide can be applied in precisely controlled concentrations, and its effects on spinal motoneurons can be observed stably with either

intra- or extracellular recording (Konishi & Otsuka, 1974). Immunohistochemical studies have shown that spinal motoneurons are surrounded by SP-containing varicosities (Barber *et al.* 1979; Hökfelt *et al.* 1975, 1977), suggesting that some SP-mediated synapses are located on spinal motoneurons. Furthermore, recent studies by Jessell *et al.* (1979) showed that the SP content in the motoneurone area of the rat spinal cord is considerably reduced after dorsal rhizotomy. Therefore, some of the SP-containing terminals on the motoneurons may originate from primary afferent neurones, although other terminals seem to have a supraspinal origin (Hökfelt *et al.* 1977; Kanazawa *et al.* 1979).

In the present study we therefore studied the effects of SP on the motoneurons of isolated rat spinal cords. We focused attention particularly on two aspects: the time course of SP action and the antagonism of baclofen to SP. Many authors using electrophoretic application techniques reported that the time course of the action of SP on central neurones is characteristically slow and long-lasting (Henry, Krnjević & Morris, 1975; Krnjević & Morris, 1974; Phillis & Limacher, 1974). In the spinal neurones of the cat, for example, Henry *et al.* (1975) reported that the excitant action of SP appeared with a delay of 15–30 s and lasted for 1–2 min after the application was stopped. In these experiments, however, the slow time course of the SP action might reflect the delayed ejection of the peptide from the micropipette (Guyenet *et al.* 1978). Concerning the effect of baclofen, Saito, Konishi & Otsuka (1975) reported that the SP-induced depolarization of rat spinal motoneurons was antagonized by baclofen [β -(4-chlorophenyl)- γ -aminobutyric acid], whereas the glutamate-induced depolarization was affected much less by the drug. However, later studies using electrophoretic techniques could not confirm the specificity of antagonism between baclofen and SP (Henry & Ben-Ari, 1976; Phillis, 1976), and it was assumed that baclofen exerts a general depressant action on central neurones (Johnston, 1978). We have therefore re-examined the effects of baclofen on the actions of SP and other drugs. A preliminary report of this work has been published (Otsuka & Yanagisawa, 1978).

METHODS

Preparation. Wistar rats of 0–3 days of age were used. Under ether anaesthesia, the spinal cord together with the vertebral column was removed and placed in a dish filled with oxygenated physiological solution at room temperature. The vertebral canal was opened under a dissecting microscope, and the spinal cord below Th 10 was isolated together with the L₃–L₅ ventral and dorsal roots. The cord was hemisected and as much as possible of the pia mater of the lumbar portion was removed. In many experiments the spinal cord was treated with collagenase (Sigma, 0.05 mg/ml) for 30 min to facilitate the removal of the pia mater.

Physiological solution. Artificial cerebrospinal fluid (CSF) of the following composition was used (mM): NaCl 138.6; KCl 3.35; CaCl₂ 1.26; MgCl₂ 1.16; NaHCO₃ 21.0; NaH₂PO₄ 0.58; glucose 10; gassed with 95% O₂ and 5% CO₂ (cf. Feldberg & Fleischhauer, 1960). In some experiments, CaCl₂ and MgCl₂ concentrations were changed as described below. The temperature of perfusion fluid was kept at 27 °C.

Perfusion system. As shown in Fig. 1A, the isolated spinal cord was placed in a 0.2 ml bath and perfused with oxygenated artificial CSF at a rate of 3–8 ml/min. For

Applying drugs for short periods, the perfusion system shown in Fig. 1 C was used in most experiments. The drug solutions were contained in syringes *a* and *b*, which were connected to syringe *c* filled with air and partly with perfusion solution. Since the reservoirs of perfusion fluids were placed at the height of about 1.3 m, there was a constant pressure in the syringes *a*, *b* and *c*. By the use of a multi-channel stimulator and electromagnetic valves, the drug solutions could be applied for a predetermined duration (0.1–10 s) at constant intervals. When cock *d* was opened and cock *e* was

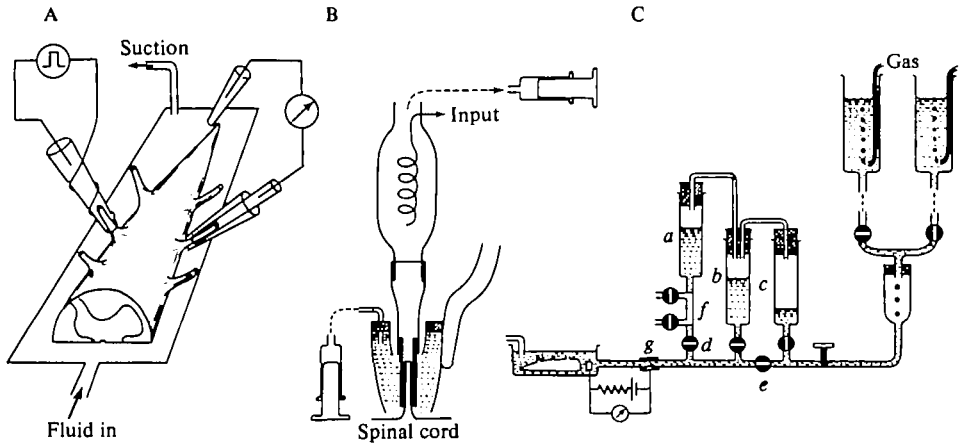


Fig. 1. Experimental set up. A, Isolated spinal cord of newborn rat and the perfusion bath. B, Extracellular recording electrode. C, Perfusion system for applying drugs. For details see text.

closed, the drug solution in syringe *a* flowed into the perfusion system. A quite reproducible amount of drug solution could be introduced into the bath and thus the constant size of responses could be recorded at relatively short intervals (1–6 min) for a long period (Figs. 7–10). When it was necessary to determine exactly the time at which the test solution entered the bath, an air bubble was placed in the tube marked *f* in Fig. 1 C, so that the test solution flow was preceded by the air bubble. Electrical resistance was recorded between the steel tubing *g* and the bath. When the air bubble passed the steel tubing, the resistance suddenly increased and then, when the air bubble entered the chamber, it decreased, thus indicating the time of entrance of the test solution (Fig. 2).

Electrodes. For stimulating the dorsal or ventral root, a suction electrode with a tip inner diameter of about $300\ \mu\text{m}$ was used. For extracellular recordings from the ventral root, the electrode shown in Fig. 1 B was used. The outer barrel of the electrode had a tip inner diameter of about $500\ \mu\text{m}$ and was filled with a mixture of liquid paraffin and Vaseline (about 1:1). The level of the liquid paraffin–Vaseline mixture could be controlled by applying pressure with the syringe connected to the outer barrel. The inner barrel was a suction electrode filled with physiological solution and connected through an Ag–AgCl wire to a preamplifier. The electrode was connected through polyethylene tubing to a short piece of glass capillary with a fire-polished orifice that had an inner diameter of 100 – $150\ \mu\text{m}$, depending on the size of the ventral root. The relative positions of inner and outer barrels could be adjusted by a

micromanipulator, and the whole assembly of the recording electrode was mounted on another micromanipulator. To get good responses to SP, it was important to dissect the ventral root close to the surface of the spinal cord and to use a capillary at the tip of the inner barrel that fitted tightly around the ventral root. After the ventral root was sucked into the inner barrel, the outer barrel was lowered and a short distance (about 1 mm or less) of the ventral root was insulated by the mixture of paraffin and Vaseline (Fig. 1B). The bath was grounded through a calomel electrode. The pre-amplifier was connected to an oscilloscope, and through a transient memory circuit to a pen recorder.

In a few experiments, intracellular recordings were made from spinal motoneurons. The motoneurons were identified by antidromic action potentials induced by ventral root stimulation. Microelectrodes filled with 2 M-K-acetate with resistance of 50–120 M Ω were used.

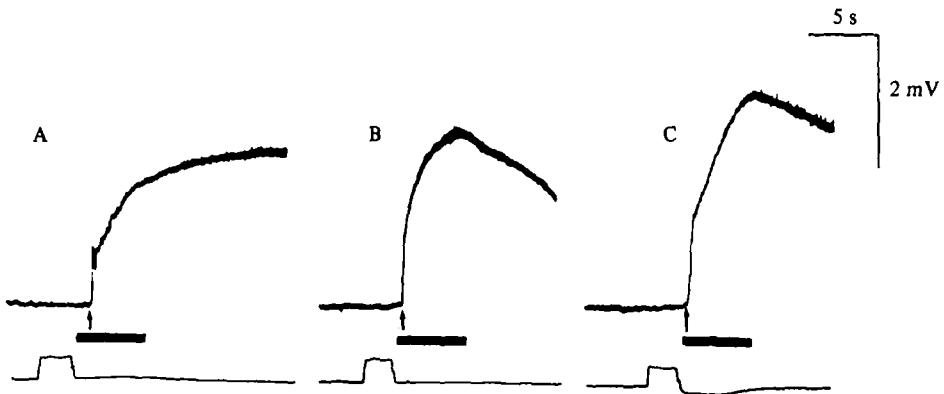


Fig. 2. Time course of actions of 8×10^{-8} M SP (A), 10^{-3} M L-glutamate (B) and 30 mM-KCl (C). Potentials were recorded extracellularly from L₄ ventral root of isolated spinal cord of 1-day-old rat. Upper traces show the potential recorded from the ventral root in normal artificial CSF. Upward deflexion shows the depolarization in this and following figures. Lower traces monitor the resistance between the steel tubing (*g*) and the bath as shown in Fig. 1C. In each record, the chamber was flushed with the drug solution during the period indicated by horizontal black bar, and arrow indicates the beginning of depolarization.

RESULTS

Time course of SP action. When SP in a concentration of 8×10^{-8} M was introduced into the chamber, the depolarization of motoneurons began with a delay of 1.1 ± 0.1 sec (mean \pm S.E.M., $n = 4$; Fig. 2A). Similarly, the depolarizations induced by L-glutamate (10^{-3} M) and KCl (30 mM) appeared with delays of 0.4 ± 0.1 and 0.2 ± 0.1 s, respectively (mean \pm S.E.M., $n = 4$; Fig. 2B and C). In the experiment shown in Fig. 3A, a lower concentration of SP (10^{-6} M) was applied for about 5 s in artificial CSF containing 1.26 mM-Ca and 3.5 mM-Mg. The depolarization appeared with a delay of about 3 s. The time from onset to peak and the half decay time were 14 and 9 s, respectively.

In order to examine whether the SP-induced depolarization is due to direct or trans-synaptic action on the motoneurons, SP (10^{-6} M) was applied in artificial CSF containing 0.1 mM-Ca and 3.5 mM-Mg, where spinal reflexes induced by dorsal root

stimulation were completely blocked (cf. Fig. 4). As shown in Fig. 3B, the size and the time course of SP-induced depolarization were similar to those in the 1.26 mM-Ca, 3.5 mM-Mg medium. Since the distance between the surface of the spinal cord and the motoneurone area is more than 100 μm , it is likely that the time course of the depolarization of motoneurons in the low-Ca medium reflects the change of SP

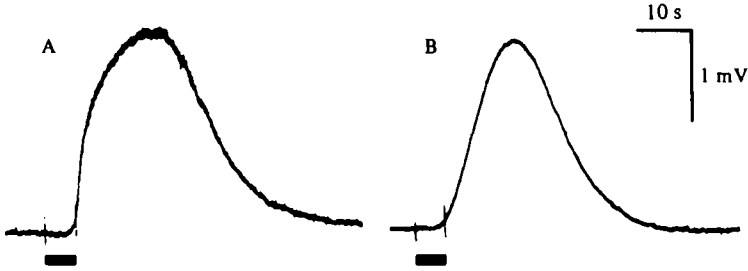


Fig. 3. Time course of the depolarizing responses to SP. Extracellular recordings from the ventral root in this and all subsequent figures. (A) In artificial CSF containing 1.26 mM-Ca and 3.5 mM-Mg; and (B) in the medium containing 0.1 mM-Ca and 3.5 mM-Mg. SP solutions (10^{-7} M) were applied to the bath during the periods indicated by horizontal black bars.

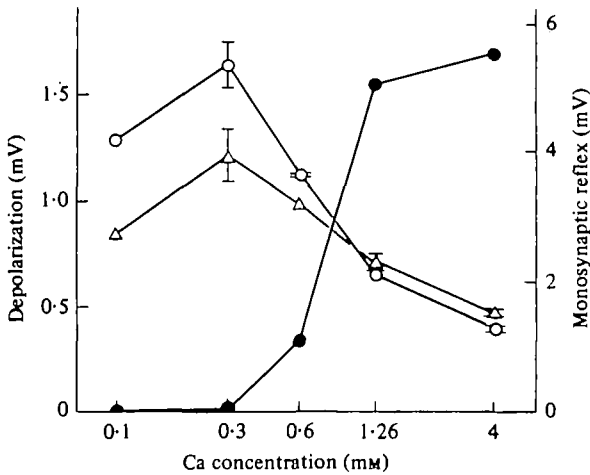


Fig. 4. Effects of Ca concentrations on the size of monosynaptic reflex (●), SP-induced depolarization (O), and L-glutamate-induced depolarization (Δ). Vertical lines represent s.e.m. ($n = 2-4$). Potential changes were recorded from L₄ ventral root and the spinal reflexes were induced by a single supramaximal volley in the corresponding dorsal root with intervals of 11 s. SP (10^{-7} M) and L-glutamate (2×10^{-4} M) were applied for 30 s periods. Mg concentration was kept at 2 mM. Abscissa: logarithmic scale.

concentration at the site of action on motoneurons (Fig. 3B). The rising phase of SP-induced depolarization in the 1.26 mM-Ca medium was steeper than that in the 0.1 mM-Ca medium, suggesting that SP activates excitatory interneurons in addition to its direct action on motoneurons.

That SP exerts a direct depolarizing action on motoneurons is further supported by the results shown in Fig. 4. When the Ca concentration in the medium was changed from 4 to 0.1 mM, keeping the Mg concentration at 2 mM, the spinal reflexes induced by dorsal root stimulation and recorded from the corresponding ventral root were

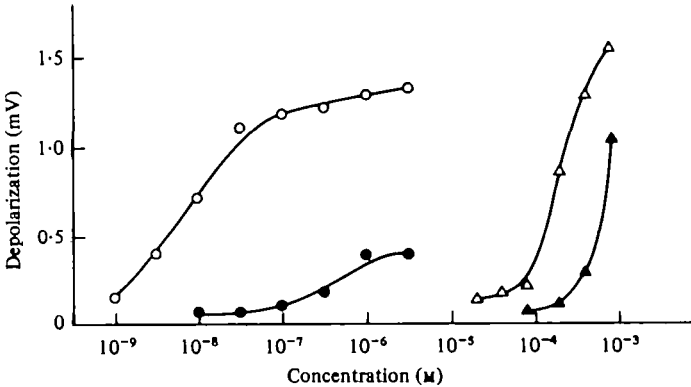


Fig. 5. Effects of TTX on SP-induced and L-glutamate-induced depolarizations. SP and L-glutamate were applied to the bath during the periods of 30 s in concentrations shown in abscissa. \circ , \bullet , Responses to SP; Δ , \blacktriangle , responses to L-glutamate. Open symbols indicate the responses in artificial CSF containing 0.1 mM-Ca and 1.6 mM-Mg and closed symbols indicate those after the addition of TTX (1.3×10^{-7} M).

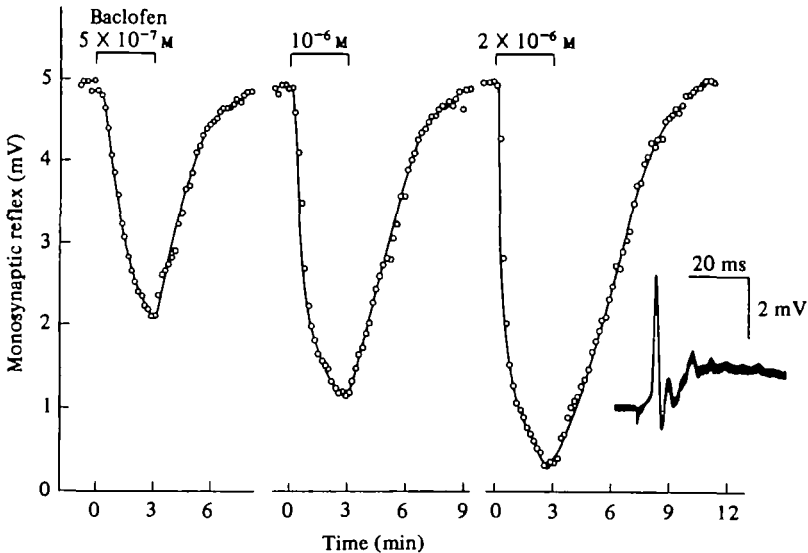


Fig. 6. Effects of baclofen on the sizes of monosynaptic reflex. Spinal reflexes were induced by a single supramaximal volley in L₄ dorsal root with intervals of 11 s and recorded extracellularly from L₄ ventral root. Baclofen was applied to the bath in three different concentrations during the periods marked in the figure. Inset shows an example of the record of spinal reflexes.

greatly depressed in 0.6 mM-Ca and abolished in 0.3 mM-Ca medium. In contrast, both SP-induced and L-glutamate-induced depolarizations were larger in 0.1 and 0.3 mM-Ca solutions than those in 1.26 mM-Ca solution. To confirm further that the synaptic transmission in the spinal cord is blocked in low-Ca medium, intracellular recordings were made from motoneurons. When the perfusion medium was changed from normal artificial CSF to the medium containing 0.2 mM-Ca and 2 mM-Mg, the excitatory postsynaptic potentials (EPSPs) induced by repetitive stimulation (10 stimuli at 100 Hz) of the dorsal root of the corresponding segment were also completely abolished in 5–10 min.

We also studied the effects of tetrodotoxin (TTX), which is another means to block synaptic transmission. When the isolated rat spinal cord was perfused with artificial CSF containing TTX (1.3×10^{-7} M), the spinal reflexes were completely abolished. Under such conditions, both SP- and glutamate-induced depolarizations were greatly depressed. The depressant effects of TTX on SP- and glutamate-induced responses were observed, however, not only in normal artificial CSF but also in the 0.1 mM-Ca medium (Fig. 5), suggesting that TTX acts directly on the motoneurons (see Discussion).

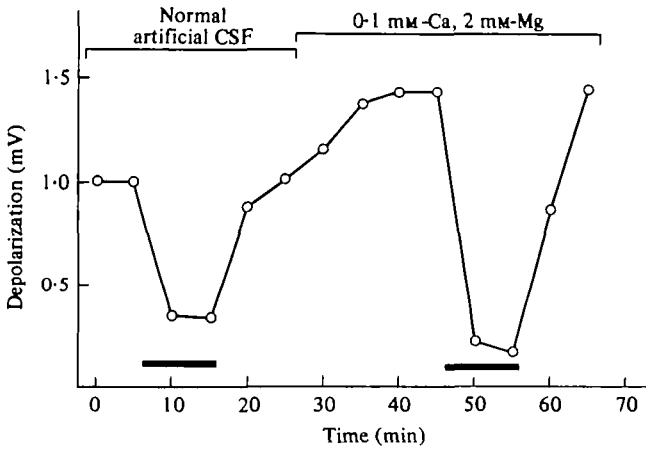


Fig. 7. Effects of baclofen (10^{-6} M) on the SP-induced responses in normal and low-Ca mediums. SP (6×10^{-7} M) was applied with pulses of 3 s duration with intervals of 5 min. The depolarizing responses were recorded from L4 ventral root. The preparation was first perfused with normal artificial CSF and then with the medium containing 0.1 mM-Ca and 2 mM-Mg. Baclofen was added during the periods indicated by horizontal black bars.

Effects of baclofen. When one of the dorsal roots (L₃-L₅) is stimulated by a single shock, the response recorded from the corresponding ventral root consists of an early spike followed by later asynchronous waves as illustrated in the inset in Fig. 6. We believe that the initial spike corresponds to the monosynaptic reflex, based on the following observation. When the nerve innervating the medial or lateral gastrocnemius muscle was stimulated and the potential was recorded intracellularly from homonymous motoneurons of newborn rats, a large synchronous EPSP could be recorded (Konishi & Otsuka, unpublished observation). Fig. 6 illustrates the effect of baclofen on the size of the monosynaptic reflex. In agreement with earlier observations (Pierau & Zimmermann, 1973; Saito *et al.* 1975) baclofen blocked the spinal monosynaptic and polysynaptic reflexes. Baclofen at 10^{-6} M reduced the size of the monosynaptic reflex by about 75%. The full effect was reached within a few minutes, and after washing, the effect disappeared with a similarly rapid time course. Intracellular recordings from motoneurons revealed that baclofen (10^{-6} M) reduced the size of the EPSP induced by single-shock stimulation of the dorsal root by about 60% without affecting appreciably the size of the resting potential or the antidromic action potential (not illustrated).

In the experiment shown in Fig. 7, the responses to SP were induced by applying

SP solution (6×10^{-7} M) with short pulses of 3 s duration in normal artificial CSF. Addition of 10^{-8} M baclofen to the medium reduced the size of SP response by about 70% and complete recovery occurred within 10 min after washing. The preparation was then perfused with artificial CSF containing 0.1 mM-Ca and 2 mM-Mg, where the SP response usually became larger. The depressant effect of baclofen on the SP response was even more marked in the low-Ca medium, suggesting that baclofen acts directly on motoneurones.

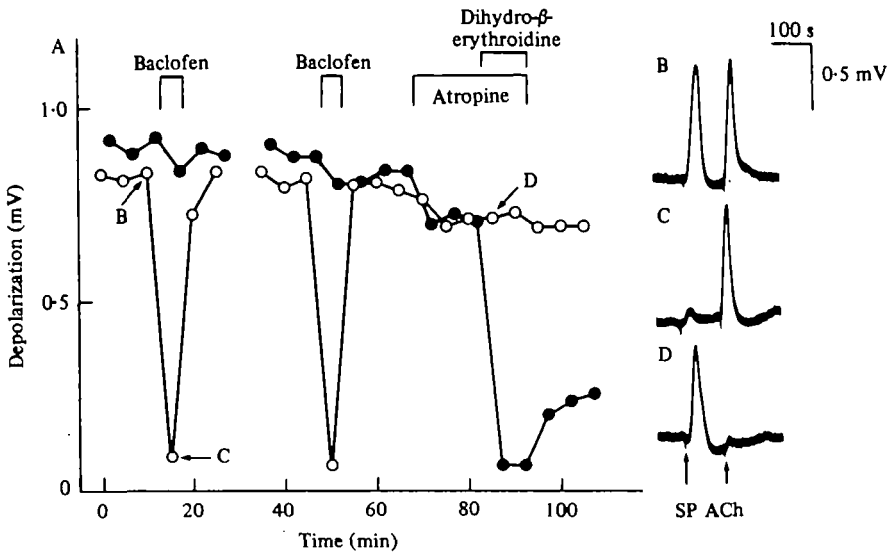


Fig. 8. Effects of baclofen (10^{-8} M), atropine (1.4×10^{-7} M) and dihydro- β -erythroidine (4×10^{-6} M) on the responses to SP and ACh. SP (3×10^{-7} M) and ACh (3×10^{-4} M) were alternately applied with pulses of 3 and 2.2 s durations respectively. The interval between SP applications was 5 min, and ACh was applied 100 s after each SP application. The preparation was perfused with artificial CSF containing 0.1 mM-Ca, 2 mM-Mg and 3×10^{-8} M prostigmine. Baclofen, atropine and dihydro- β -erythroidine were added to the medium during the periods indicated in the figure. (A), O, The response to SP; ●, the response to ACh. (B-D) The sample records in control solution, after the addition of baclofen, and after the addition of atropine and dihydro- β -erythroidine; the times when the records (B), (C) and (D) were taken are shown by arrows in (A). In each record the first response was induced by SP and the second was induced by ACh.

To test whether the antagonism between baclofen and SP is selective or not, the effects of baclofen on other agonists were examined. As a routine procedure, each agonist that produced depolarization of motoneurones was given by pulse application while the preparation was perfused with artificial CSF containing 0.1 mM-Ca and high Mg (1.6–2.0 mM). In most experiments baclofen was given in a concentration of 10^{-6} M, and the responses to SP and one of the agonists were alternately recorded at intervals of 1–6 min. In choosing the durations of pulses for applying SP and other agonists, care was taken so that two alternately given drugs gave approximately same sizes of submaximal responses.

In the experiment shown in Fig. 8, acetylcholine (ACh) and SP were applied alternately in the presence of prostigmine (3×10^{-8} M). After adding baclofen, SP response was reduced by about 90% whereas the response to ACh was reduced by only 6–8%. On the other hand, when the preparation was perfused with the solution

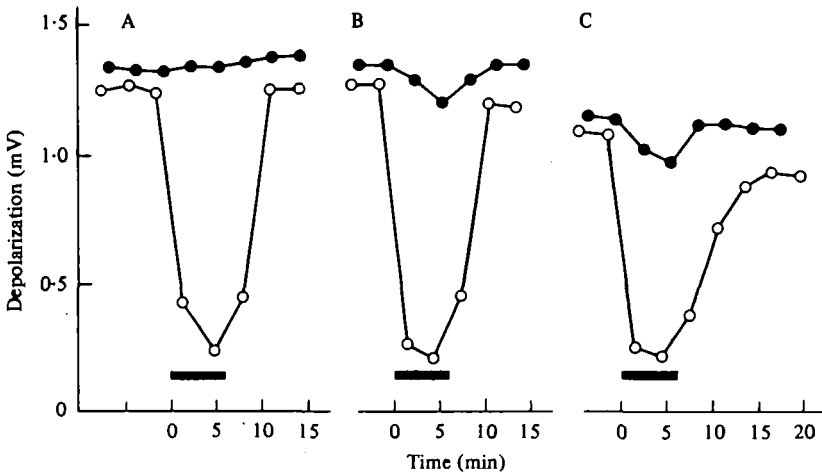


Fig. 9. Effects of baclofen (10^{-4} M) on the responses to SP, glycine, GABA and L-glutamate. The responses to SP and to one of the three amino acids were recorded alternately in artificial CSF containing 0.1 mM-Ca and 2 mM-Mg. Baclofen (10^{-4} M) was added during the period indicated by horizontal black bars. \circ , Response to SP; \bullet , response to glycine in A, to GABA in B, and to L-glutamate in C. The concentrations and the periods of application of drugs were as follows: SP 3×10^{-7} M, 3 s; glycine 10^{-3} M, 1 s; GABA 2×10^{-4} M, 1.5 s; and L-glutamate 10^{-3} M, 2.8 s. The interval between SP applications was 3 min, and one of the amino acids was applied 100 s after each SP application.

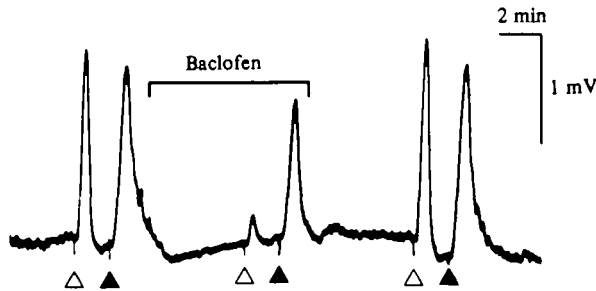


Fig. 10. Effects of baclofen (10^{-4} M) on the responses to SP (Δ) and noradrenaline (\blacktriangle) in artificial CSF containing 0.1 mM-Ca and 1.6 mM-Mg. SP (3×10^{-7} M) and noradrenaline (4×10^{-4} M) were alternately applied with pulses of 2 and 1.2 s durations respectively. Baclofen was added during the period indicated in the figure.

containing atropine (1.4×10^{-7} M) and dihydro- β -erythroidine (4×10^{-6} M) the ACh response was almost completely abolished, whereas the SP response was reduced only slightly or was not affected. These cholinergic blocking agents in the concentrations given in Fig. 8 did not affect the spinal reflexes recorded from the ventral root.

In the isolated rat spinal cord, L-glutamate, GABA, and glycine produced depolarization of motoneurons with a relatively fast time course (Evans, 1978; Konishi, Saito & Otsuka, 1975). As shown in Fig. 9, the responses to these amino acids were recorded alternately with the SP responses. Baclofen again markedly and reversibly reduced the SP response. By contrast, the response to glycine was not affected and the responses to GABA and glutamate were only slightly reduced by baclofen.

Table 1. *Effects of baclofen (10^{-6} M) on responses to SP and various depolarizing agents*

(Experiments were carried out in artificial CSF containing 0.1 mM-Ca and 1.6–2.0 mM-Mg. Extracellular recordings from the ventral root. Prostigmine (3×10^{-6} M) was added when ACh responses were recorded. In most experiments, the responses to SP and one of the depolarizing drugs were alternately recorded as shown in Figs. 8–10. Intervals of application of drugs were 1–6 min.)

Drug	Concentration (M)	Pulse duration (s)	Depression by baclofen* (%)	n
Substance P	3×10^{-7}	1–4	83 ± 2.0	15
Eledoisin-related peptide	5×10^{-7}	3	82	1
L-Glutamate	10^{-3}	0.7–3.5	18 ± 2.7	5
GABA	2×10^{-4}	0.5–1.5	10 ± 1.5	3
Glycine	10^{-3}	0.2–1.5	0.7 ± 0.4	3
Acetylcholine	3×10^{-4}	2–3	5 ± 2.1	3
Noradrenaline	10^{-4}	1–3	22 ± 4.4	5

* Mean \pm S.E.M.

n, Number of experiments.

Fig. 10 shows the effects of baclofen on the responses to noradrenaline and SP. Noradrenaline also produced a depolarizing response of relatively slow time course. Among the depolarizing drugs examined in the present study, noradrenaline was the most susceptible to the depressant action of baclofen. However, the depression of noradrenaline response by baclofen was still much less than the depression of SP response (cf. Table 1). Phentolamine (7×10^{-6} M), on the other hand, depressed the noradrenaline response by about 80% without affecting the SP response and the spinal reflexes.

Table 1 summarizes the effects of baclofen (10^{-6} M) on the responses to SP and other depolarizing drugs as observed in artificial CSF containing 0.1 mM-Ca and 1.6–2.0 mM-Mg. It is clear that baclofen antagonizes the depolarizing action of SP and eledoisin-related peptide (Lys-Phe-Ile-Gly-Leu-Met-NH₂) in a selective manner. However, the specificity of the antagonism between baclofen and SP is only relative. Thus the responses to glutamate and noradrenaline were also depressed by baclofen.

DISCUSSION

The depolarization of spinal motoneurons induced by bath-applied SP and L-glutamate began with delays of 1.1 and 0.4 s, respectively. In the lumbar spinal cord of 1-day old rats, it is likely that both SP and L-glutamate act on spinal neurones after traversing the white matter of more or less 100 μ m thickness. In fact the delay of 0.4 s for the response to glutamate probably represents the time needed for the amino acid to reach the site of action, since glutamate, when applied iontophoretically, is known to produce a depolarization of spinal neurones virtually without delay (Takahashi, 1978). Furthermore, the diffusion constant of SP is probably much smaller than that of glutamate. These considerations lead to the conclusion that the real delay time for SP-induced depolarization is shorter than several hundred ms.

this connexion, Vincent & Barker (1979) reported the SP-induced rapidly depolarizing responses of cultured spinal neurones.

The effects of SP and baclofen described in the present study can be explained by the assumption that in the 0.1 mM-Ca medium the SP-induced depolarization of motoneurones results from the direct action of SP, whereas the contribution of SP's transsynaptic action is minor. In that case the site of action of baclofen in the low-Ca medium must also be on the motoneurones. That SP acts directly on motoneurones is consistent with the presence of SP-containing varicosities around spinal motoneurones and their dendrites as demonstrated by immunohistochemical studies (Barber *et al.* 1979; Hökfelt *et al.* 1975, 1977). These studies have further shown that most of the SP-positive terminals in the spinal cord form axo-dendritic synapses. Although the mechanisms of the depressant action of TTX on SP-induced depolarization in normal or low-Ca medium are not clear, a possible explanation is that an increase of Na-permeability is involved in the depolarization produced by SP.

The depolarizing action of SP on spinal motoneurones was markedly depressed by baclofen. Although the mechanism of the action of baclofen is unknown, the antagonism between baclofen and SP showed a considerable degree of selectivity in the low-Ca medium where the synaptic transmission was blocked. Furthermore, there is evidence that baclofen blocks certain types of synapses selectively. According to the recent study of Kato, Waldmann & Murakami (1978), systemic administration of baclofen in the cat blocks the primary afferent transmission in the spinal cord without affecting other types of synaptic transmission, such as the excitation of spinal motoneurones produced by stimulation of the pyramidal tract, reticular formation, and cuneiform nucleus. The excitation of Renshaw cells elicited by antidromic ventral root stimulation was also unaffected by baclofen (Benecke & Meyer-Lohmann, 1974), which is consistent with the present observation that baclofen did not appreciably affect the ACh-induced depolarization of motoneurones. Two other excitatory pathways were recently reported to be blocked by baclofen. Kanazawa & Yoshida (1978) found that striatal stimulation produced an excitatory effect on nigral cells in the cat treated with picrotoxin and that this excitatory effect was abolished by systemic administration of baclofen. In contrast, the inhibitory effect on nigral cells produced by striatal stimulation in the normal cat was not affected by baclofen (Olpe *et al.* 1977). Sastry (1978) reported that electrophoretic administration of baclofen blocked the excitatory effect on the interpeduncular nucleus cells produced by habenular stimulation. There is neurochemical evidence that SP is involved in both striato-nigral and habenulo-interpeduncular pathways (Kanazawa *et al.* 1977; Mroz, Brownstein & Leeman, 1976). Therefore the blocking action of baclofen on some of the primary afferent, striato-nigral, and habenulo-interpeduncular synapses may be due to the antagonism of baclofen to SP that is released as a transmitter. However, it is to be noted that the specificity of baclofen is only relative. Furthermore, the drug might have multiple sites of action. For example, baclofen may be an antagonist to a certain group of transmitters that includes SP. In addition, baclofen might have a presynaptic action (see below).

The antagonism between baclofen and SP was extensively studied using electrophoretic techniques. Although these studies confirmed that baclofen depressed the excitatory effect of SP on central neurones, the effects of ACh and glutamate were

also depressed by the drug (Henry & Ben-Ari, 1976; Phillis, 1976). Therefore it was concluded that the antagonism of baclofen to SP is not specific, but is due to a general depressant action (Johnston, 1978). The discrepancies between the results of electrophoretic studies and the present results may be due to different modes of administration. Fox *et al.* (1978) reported that the effects of baclofen administered by electrophoresis are entirely different from those of systemic administration. It is possible that the local concentration of baclofen is much higher in electrophoretic application than in systemic administration.

A possibility that seems remote but cannot be ruled out from the present experiments is that the SP-induced depolarization of spinal motoneurons in low-Ca medium is mostly due to a release of excitatory transmitters acting on motoneurons that is by some unknown reason resistant to the lowering of external Ca concentration. In that case the site of action of baclofen may be postsynaptic on interneurons or presynaptic on SP-activated nerve terminals. For further elucidation of mechanisms of the actions of SP and baclofen, simple systems such as cultured spinal neurons will be useful.

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ADDENDUM

Recent experiments on mesenteric ganglia of the guinea pig have provided further support for a transmitter role of SP (S. Konishi, A. Tsunoo & M. Otsuka, *Proc. Japan Acad.* **55** B (1979), 525). The results are in close parallel to the recent findings of Kuffler and his colleagues indicating that a LHRH-like peptide is the transmitter that initiates the late slow EPSP in frog sympathetic ganglia (Kuffler, this volume; Y. N. Jan, L. Y. Jan & S. W. Kuffler, *Proc. natn. Acad. Sci. U.S.A.* **76** (1979), 1501). Our findings are summarized as follows. (1) The inferior mesenteric ganglia of the guinea pig contains a large amount of SP (1300 pg/mg protein). Seven days after the ligation of the lumbar splanchnic and intermesenteric nerves, the SP content of the inferior mesenteric ganglia is reduced to a quarter of the control and an accumulation of SP occurs in the proximal segment of the ligated nerves. (2) SP is released from the mesenteric ganglia in solutions containing 80 mM potassium. This release is five times above the 'resting' release and is calcium-dependent. (3) Bath-application of SP (0.1–5 μM) induces in mesenteric ganglion cells a depolarization which abolishes the slow EPSP. (4) The SP-induced depolarization of the ganglion cells is accompanied by changes in membrane conductance as well as in after-hyperpolarization following action potentials, and these changes are similar to those associated with the slow EPSP. (5) The action of serotonin which also depolarizes our ganglion cells is antagonized by methysergide (5 μM) which, however, does not affect the slow EPSP or the SP-induced depolarization.

Several of our findings on the mesenteric ganglia, therefore, resemble results in the spinal cord.