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PLATEAU POTENTIALS IN α-MOTONEURONES INDUCED BY INTRAVENOUS INJECTION OF L-DOPA AND CLONIDINE IN THE SPINAL CAT

BY BERNARD A. CONWAY*, HANS HULTBORN†, OLE KIEHN and ISABELLE MINTZ‡

From the Department of Neurophysiology, The Panum Institute, University of Copenhagen, Blegdamsvej 3C, DK-2200 Copenhagen N, Denmark

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SUMMARY

1. Intracellular recordings were made from lumbar α-motoneurones in unanaesthetized decerebrate acute spinal cats. The response of motoneurones to direct current pulse injection or synaptic excitation was investigated following intravenous injection of L-β-3,4-dihydroxyphenylalanine (L-DOPA, 20–120 mg/kg) alone, nialamide (10–50 mg/kg) and L-DOPA or clonidine (0·5–1 mg/kg).

2. The response properties of motoneurones were tested with rectangular and triangular current waveforms. Before L-DOPA treatment motoneuronal firing during a rectangular current pulse is characterized by an initial high firing frequency which rapidly decreases to a lower steady-state firing which is maintained only for the duration of the pulse. Following administration of L-DOPA an acceleration in firing frequency is apparent following the initial adaptation seen with rectangular current pulses. A transient after-depolarization or an after-discharge often followed the termination of the pulse. The frequency–current relation in response to a triangular current injection changed from a clockwise to a counter-clockwise hysteresis after L-DOPA treatment (i.e. after L-DOPA the firing frequency was higher for any given current during the descending phase than during the ascending phase of the triangular waveform).

3. Firing acceleration during and self-sustained firing after rectangular current pulses and counter-clockwise hysteresis of firing frequency with triangular current pulses are causally related to the presence of plateau potentials, which can be directly visualized after inactivation of the spikes. Plateau potentials in motoneurones could be generated by short-lasting intracellular depolarizing current pulses or brief excitatory synaptic inputs and terminated by short-lasting hyperpolarizing current pulses or brief inhibitory synaptic inputs. Plateau potentials were demonstrated in flexor and extensor motoneurones.

4. All bistable properties described in the preceding paragraphs following L-DOPA

* Present address: Institute of Physiology, University of Glasgow, Glasgow G12 8QQ.
† To whom reprint requests should be sent.
‡ Present address: Laboratoire de Neurobiologie Cellulaire, Institut Pasteur, 75015 Paris, France.

administration could also be seen after administration of the α-receptor agonist clonidine.

5. Slow rhythmic oscillations of the membrane potential (7·5–10 Hz) were seen superimposed on plateau potentials in a few cells after administration of L-DOPA and clonidine. The oscillations had an amplitude in the range 10–20 mV and represent the expression of an intrinsic property of the motoneurone.

6. It is demonstrated that plateau potentials in the motoneurones contribute to the late long-lasting reflexes observed in L-DOPA-treated spinal cats.

7. It is concluded that L-DOPA (and clonidine) change the response properties of the motoneurones in an analogous way to 5-hydroxy-DL-tryptophan (5-HTP). Nevertheless, it is argued that 5-HTP and L-DOPA (by releasing serotonin and noradrenaline respectively) act on separate receptors, which may share common second messenger and effector mechanisms. The functional implications of plateau potentials in the motoneurones are discussed.

INTRODUCTION

In the preceding paper Hounsgaard, Hultborn, Jespersen & Kiehn (1988) demonstrated that spinal motoneurones in the decerebrate cat could generate plateau potentials in response to excitatory synaptic inputs or to brief depolarizing current pulses. The underlying neuronal properties seemed to be contingent upon an active descending serotonergic raphe–spinal innervation of the motoneurones. The possibility of evoking plateau potentials thus disappeared after an acute spinal transection but reappeared after intravenous injection of the serotonin precursor 5-HTP.

In addition to a descending innervation of the spinal cord from serotonergic neurones arising from the raphe nuclei a noradrenergic innervation from the locus coeruleus and neighbouring nuclei has been identified (Dahlström & Fuxe, 1964, 1965; summarized by Björklund & Skagerberg, 1982). Serotonin and noradrenaline appear to have very similar actions on spinal reflex transmission (Andén, Jukes & Lundberg, 1964; see further in Discussion). It is therefore of interest to investigate in the acute spinal cat the effect that L-DOPA (which causes synthesis and release of noradrenaline; for references see Baldissera, Hultborn & Illert, 1981) may have on the motoneurones themselves and compare these actions with those previously described for 5-HTP (Hounsgaard *et al.* 1988).

It will be shown that L-DOPA, like 5-HTP, can induce plateau potentials in motoneurones. This effect could be mimicked by intravenous injection of the α-receptor agonist clonidine. Furthermore, it will be demonstrated that plateau potentials contribute to the prolonged discharge of the late and long-lasting flexor reflexes seen in L-DOPA-treated spinal animals (Andén, Jukes, Lundberg & Vyklický, 1966*b*). Some of the results presented in this paper have been published in abstract form (Conway, Hultborn & Mintz, 1985).

METHODS

Preparation. The results reported in this paper are based on experiments performed on twenty-one adult cats. The general procedures including anaesthesia, surgical interventions and maintenance of the preparation are as described in an accompanying paper (Crone, Hultborn, Kiehn, Mazieres & Wigström, 1988). Only a brief account will be given here.

Lengths of the nerves to the following muscles were dissected free on the left (ipsilateral) side: posterior biceps–semitendinosus, anterior biceps–semimembranosus, medial gastrocnemius, lateral gastrocnemius–soleus, plantaris–flexor digitorum longus, tibialis anterior, peroneus brevis and tertius, sartorius and quadriceps. The sural nerve and the superficial peroneal nerve were used to stimulate cutaneous afferents. In the right limb (contralateral) the nerves to the hamstring muscles, triceps surae group and quadriceps were dissected free along with a length of the sural nerve.

Recording and stimulation. Intracellular recordings from antidromically identified motoneurones and gross neuronal recordings from cut muscle nerves (electroneurograms, ENGs) were made in accordance with the descriptions provided in the second paper of this series (Hounsgaard *et al.* 1988).

Drugs. A 1 % solution of L-DOPA (L-β-3,4-dihydroxyphenylalanine; Sigma/Fluka) was obtained by dissolving the substance in 0·9 % NaCl under warming and stirring. The solution was given intravenously over a 5–10 min period in a dose ranging from 20 to 120 mg/kg. In the final eleven experiments the methyl ester of L-DOPA (Sigma) was used. This ester is readily dissolved in saline and the volume of fluid required to attain a certain dosage of L-DOPA can be greatly reduced.

If L-DOPA is given alone the resulting flux of noradrenaline from nerve terminals is to a large extent countered by monoamine oxidase activity and by the reuptake of noradrenaline by the terminals; therefore, large doses (100–120 mg/kg) are required. However, the large volume of fluid required with such doses usually leads to mechanical instabilities during intracellular recordings. In order to overcome this problem the effectiveness of L-DOPA was increased (and hence the dose required reduced) by either pre-treatment with a monoamine oxidase inhibitor (nialamide) or a noradrenergic uptake blocker (maprotiline, Ludiomil). Nialamide (10–50 mg/kg; Sigma) was given intravenously 1–2 h prior to L-DOPA (20–100 mg/kg) in nine experiments while maprotiline (3 mg/kg; a gift from Ciba Geigy, Copenhagen) was given immediately prior to L-DOPA in one experiment. It has been reported that some time after nialamide treatment there may be an effect on spinal reflexes (Andén *et al.* 1964) conceivably resulting from a potentiation of spontaneously released noradrenaline and serotonin. In order to discount a significant contribution from serotonin upon motoneuronal and reflex studies L-DOPA was used without nialamide and maprotiline pre-treatment in seven experiments while in four experiments the α-agonist clonidine (0·5–1 mg/kg, I.V.; Sigma) was administered. In general no significant differences could be detected in the results from animals pre-treated with nialamide and those which were not.

RESULTS

(1) *Response properties of motoneurones after I.V. administration of L-DOPA*

In the preceding paper (Hounsgaard *et al.* 1988) it was demonstrated that large differences exist in the relationship between motoneuronal firing frequency and stimulus current intensity (*f–I* relation) prior to and following 5-HTP treatment in the acute spinal cat. In the steady state the slope of the *f–I* relationship for a particular motoneurone was shown to increase following 5-HTP treatment. Similarly, the response to triangular current pulses also changed systematically with 5-HTP. Prior to 5-HTP treatment motoneurones displayed a clockwise hysteresis in the *f–I* relation while following 5-HTP treatment a counter-clockwise hysteresis became evident on the presentation of a triangular current waveform. Thus for any given current intensity the motoneurone fired with a higher instantaneous frequency on

the descending phase of the waveform than on the ascending phase. It was argued that these phenomena and the acceleration seen during rectangular current pulses are all dependent on the presence of plateau potentials in the motoneurones (Hounsgaard *et al.* 1988). In the present investigation the response of motoneurones

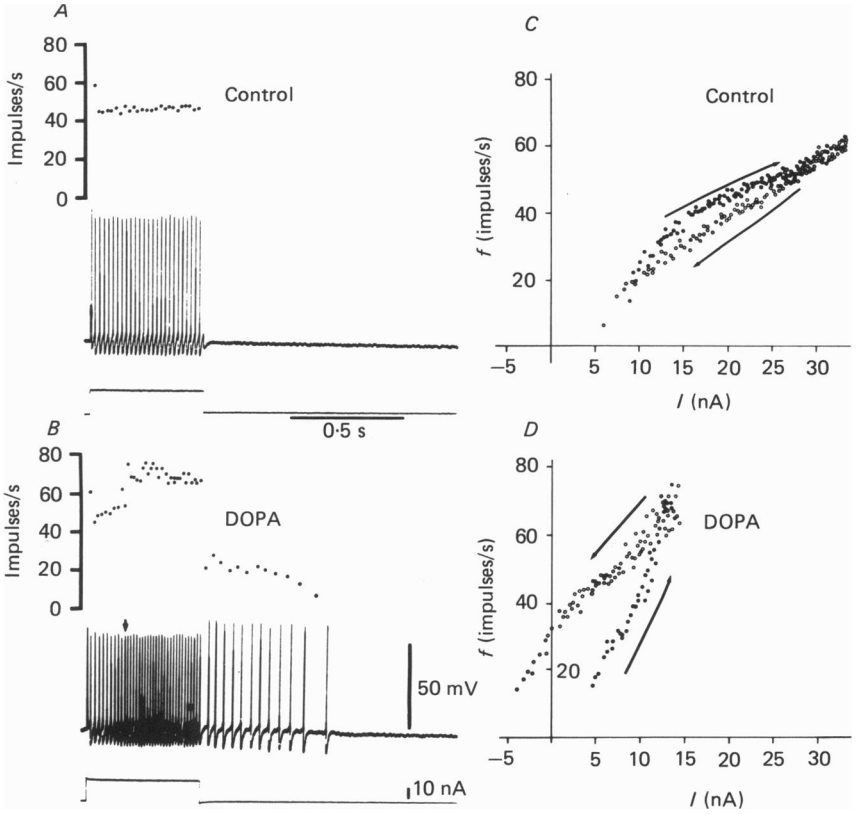

Fig. 1. Response of a posterior biceps–semitendinosus motoneurone to a rectangular (*A* and *B*) and a triangular (*C* and *D*) current pulse injection. *A* and *C*, acute spinal cat pretreated with nialamide, 20 mg/kg. *B* and *D*, same cell 18 min after I.V. injection of L-DOPA, 30 mg/kg. Instantaneous firing frequency, intracellular and injected current records are illustrated in *A* and *B*. The amplitude of the current pulse was +18 nA in *A* and +17 nA in *B*. The intracellular signal was passed through a 25 Hz filter for reproduction. In *C* and *D* the instantaneous firing frequency is plotted against injected current. The direction of the arrows indicates the ascending (●) and descending (○) phase of the triangular waveform. The slope of current injected is +7·9 nA/s (ascending and descending phases) in *C* and *D*. *C*, before L-DOPA, the frequency–current relation shows a clockwise hysteresis. *D*, after L-DOPA, the frequency–current relation shows a counter-clockwise hysteresis.

to rectangular and triangular current pulses were used as a means of testing for the presence of plateau potentials.

Response to square pulses. In the acute spinal cat a depolarizing current pulse evokes a train of action potentials with an initial phase of adaptation followed by a stable firing frequency which is only maintained for the duration of the pulse (cf. Fig.

1*A*). This firing pattern was seen in all neurones tested in acute spinal cats prior to pharmacological treatment (ten cells from five cats; see also Hounsgaard *et al.* 1988) or acute spinal cats pre-treated with nialamide alone (twelve cells from six cats). This response pattern to rectangular current pulses is similar to that previously described for deeply anaesthetized rats and cats with intact spinal cords (Granit, Kernell & Shortess, 1963; Kernell, 1965). However, following the injection of L-DOPA (Fig. 1*B*) motoneurones no longer behave in the above manner to a rectangular current pulse. Rather than displaying a steady firing after an initial

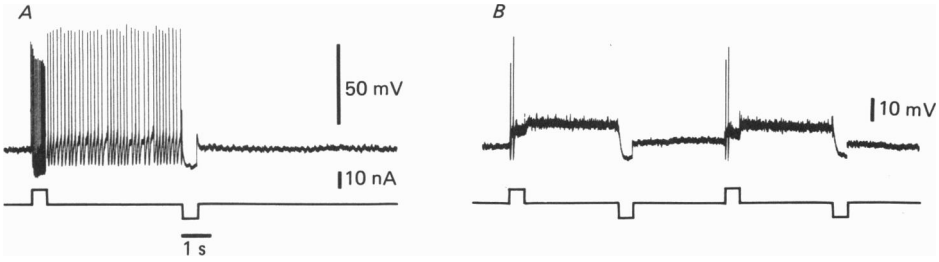

Fig. 2. Sustained shift in excitability triggered by depolarizing and hyperpolarizing intracellular current injection. *A* and *B*, upper traces illustrate intracellular recordings from a medial gastrocnemius motoneurone (acute spinal cat treated with L-DOPA, 100 mg/kg), lower traces show the timing and amplitude of current pulse injection. The steady bias current was +5 nA and −2 nA in *A* and *B* respectively. Note different voltage calibration in *A* and *B*.

adaptation during the pulse (cf. Fig. 1*A*) the cell displays a sudden frequency jump (or acceleration) to a higher frequency of discharge which is maintained until the termination of the pulse. Following the termination of the pulse the cell continues to fire (now at a lower frequency) for some time (Fig. 1*B*). This jump in firing frequency and the after-discharge seen following the pulse can be associated with the expression of a plateau potential in the motoneurone (see Hounsgaard *et al.* 1988). The duration of the after-discharge was sensitive to the holding potential (see later).

Response to triangular current pulses. Figure 1*C* and *D* shows the frequency response of the same flexor motoneurone illustrated in Fig. 1*A* and *B* to a long-lasting triangular current pulse presented before (*C*) and after (*D*) L-DOPA treatment. Prior to L-DOPA administration the cell displays a clockwise hysteresis in its *f–I* relation as would be expected from the late adaptation described by Kernell & Monster (1982). However, following injection of L-DOPA the *f–I* relation displays a conspicuous counter-clockwise hysteresis (Fig. 1*D*). It can also be seen in Fig. 1*D* that during the ascending phase of the current pulse (at around 40 Hz firing frequency) there is a marked acceleration (increased slope) of the firing frequency of the cell. This is not seen prior to L-DOPA. Furthermore, reference to the two plots illustrated in Fig. 1*C* and *D* indicates that following L-DOPA injection the cell's overall excitability has increased (i.e. the amount of current required to fire the cell at any given frequency is reduced after L-DOPA treatment).

Sustained shift in membrane potential. Figure 2*A* illustrates a self-sustained motoneuronal discharge triggered by a brief depolarizing current pulse. This

sustained firing is only terminated by a short hyperpolarizing pulse. Excessive depolarization of the cell leads to an inactivation of the fast sodium spikes whereupon brief depolarizing current pulses will initiate long-lasting potential shifts of about 10 mV (Fig. 2*B*). The holding potential of the motoneurone was critical for the generation of self-sustained plateau potentials. From hyperpolarized levels the response to a brief current pulse is seen as a transient after-discharge, while from a more depolarized holding potential the response to the same depolarizing pulse is seen as a self-sustained all-or-none response (Fig. 2*A* and *B*).

TABLE 1. Summary of the number of different extensor and flexor motoneurones tested for bistability following administration of L-DOPA and clonidine. Cells in the L-DOPA columns were either cats treated with L-DOPA alone or in conjunction with nialamide. Compare Table 1 in Hounsgaard *et al.* (1988)

	DOPA		Clonidine	
	Positive	Negative	Positive	Negative
Triceps surae	21	5	4	2
Semimembranosus–anterior biceps	1	2	1	0
Plantaris–flexor digitorum longus	5	4	—	—
Posterior biceps–semitendinosus	2	1	3	1
Tibialis anterior	8	2	4	2
Peroneus brevis–tertius	1	0	—	—
Total	38	14	12	5

As with 5-HTP-treated acute spinal cats (Hounsgaard *et al.* 1988) the plateau potentials seen following L-DOPA infusion can be triggered by short-lasting excitatory and inhibitory synaptic inputs respectively (see section (4) on 'late reflexes').

Positive signs of bistability were seen in thirty-eight cells out of a sample of fifty-two good stable intracellular recordings made following L-DOPA treatment. Table 1 provides a summary of the distribution of positive cells. No motoneurones recorded prior to L-DOPA treatment were seen to display bistability.

(2) *Response properties of the motoneurones after intravenous injection of clonidine*

It is intriguing that plateau potentials in motoneurones are uncovered after intravenous injection of both 5-HTP (Hounsgaard *et al.* 1988) and L-DOPA in the acute spinal cat. A recent study suggests that L-DOPA may release serotonin from central terminals (Commissiong & Sedgwick, 1979). To exclude the possibility that L-DOPA's effect was primarily mediated by serotonin we have used clonidine (an α-receptor agonist that crosses the blood–brain barrier) and which in relatively high doses stimulates postsynaptic receptors (Andén, Corrodi, Fuxe, Hökfelt, Hökfelt, Rydin & Svensson, 1970; Svensson, Bunney & Aghajanian, 1975; Andén, Grabowska & Strömbom, 1976).

The upper traces in Fig. 3*A* and *B* show intracellular recordings from an extensor motoneurone after I.V. injection of clonidine (0·5 mg/kg). Sustained repetitive firing was initiated and terminated by short-lasting intracellular current pulses (Fig. 3*A a*).

Each spike was followed by a pronounced delayed depolarization (Fig. 3*Ab*) of the 'hump' type (Granit, Kernell & Smith, 1963; Kernell, 1964; Calvin & Schwindt, 1972). Later during the recording, when the plateau was initiated by a synaptic excitation resulting from stimulation of the contralateral hamstring nerve (Fig. 3*Ba*), a second spike appeared on the peak of the delayed depolarization (Fig. 3*Bb*).

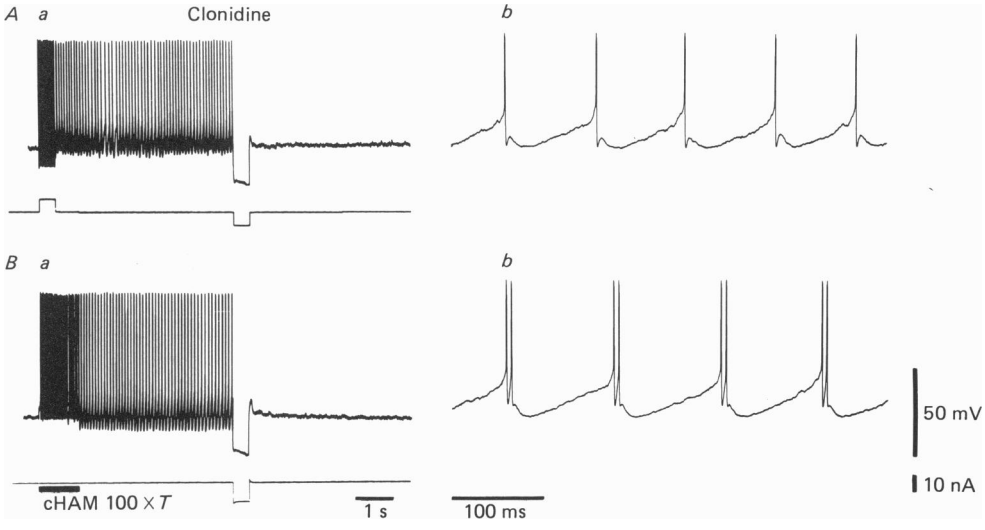

Fig. 3. Response properties of motoneurones following intravenous injection of clonidine. *Aa* and *Ba*, upper traces intracellular recordings from a medial gastrocnemius motoneurone; lower traces injected current. The timing of the afferent volley from the contralateral hamstring group (cHAM $100 \times T$, *Ba*) is indicated by a bar below the intracellular recording. Clonidine (0·5 mg/kg) was given intravenously before the recordings. *Ab* and *Bb* are extended parts of *Aa* and *Ba*. The steady bias current was +4 nA in *A* and *B*.

Clonidine's action on motoneuronal firing properties was tested in four cats. In total twelve cells from a sample of seventeen stable recordings demonstrated bistable properties (see Table 1). In addition five of these motoneurones tended to fire double spikes.

In a series of preliminary tests (seven cats) noradrenaline was applied directly to the spinal cord via a thin cannula inserted longitudinally between the dura and the ventral surface of the spinal cord. The cord was flushed with small amounts of fluid containing noradrenaline (10–30 mM). These experiments were often carried out towards the end of the experiments reported in this paper after the effects of L-DOPA had disappeared (as monitored by an absence of 'late reflexes'; see section (4)).

In five cats good 'late reflexes' monitored in the peripheral nerve developed after local application of noradrenaline to the spinal cord. In one cat long-lasting rhythmic locomotor-like activity was induced. This was blocked by I.V. injection of phentolamine (1·5 mg/kg), an α-receptor blocker. Following local application of noradrenaline stable penetrations of motoneurones became difficult. Despite this, bistable behaviour was recorded in one motoneurone. These results further strengthened the idea that 5-HTP and L-DOPA exert their actions via serotonergic and noradrenergic receptors respectively in the spinal cord even though they induce the same changes in the response pattern of the motoneurones.

(3) *Rhythmic oscillations of membrane potential in the α-motoneurone induced by* L-*DOPA and clonidine*

In a few motoneurones injection of L-DOPA or clonidine in addition to plateau potentials induced slow regular oscillations of the membrane potential. These oscillations were seen in nine motoneurones recorded in two L-DOPA- and two

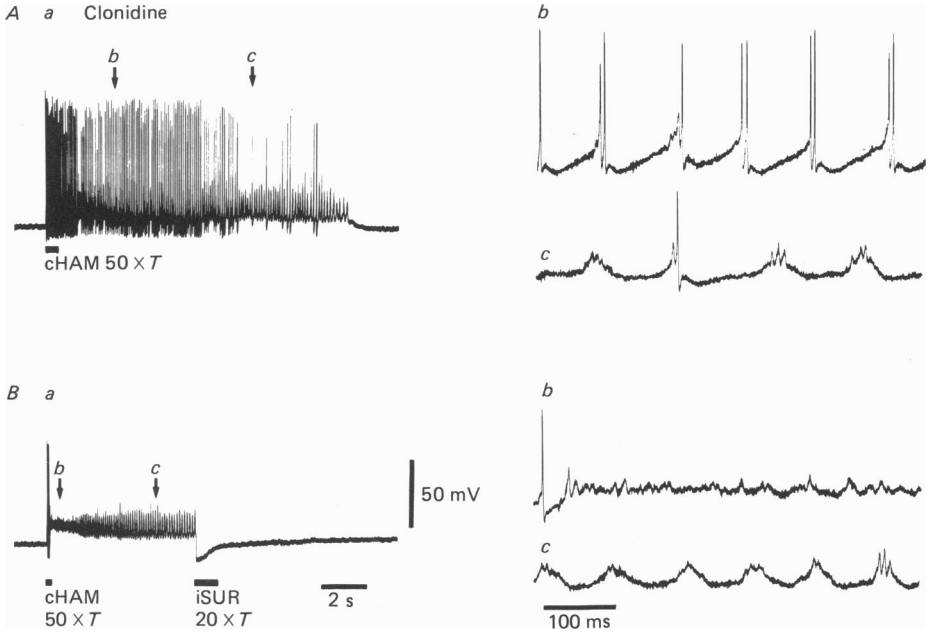

Fig. 4. Rhythmic oscillations of membrane potential in the α-motoneurones induced following clonidine administration. *Aa* and *Ba*, intracellular recordings from a plantaris–flexor digitorum longus motoneurone, after I.V. injection of clonidine (0·5 mg/kg). *Ab*, *Ac*, *Bb* and *Bc* are extended parts of *Aa* and *Ba* (marked by arrows). The sustained discharge in *Aa* was initiated by a short train of stimuli from the contralateral hamstring nerve (cHAM, 50 × *T*) as indicated by the bar below the intracellular record. The spike-generating mechanism inactivated and revealed slow regular oscillations of the membrane potential. *Ab*, shows double spiking triggered at the peak oscillations, which are seen more clearly in *Ac*. *Ba*, same cell from a more depolarized holding potential. The plateau potential, also seen in *Bb*, was initiated by an afferent volley in the contralateral hamstring nerve (cHAM, 50 × *T*) and terminated by a train of stimuli in ipsilateral sural nerve (iSUR, 20 × *T*) as indicated by the bar. The slow oscillations superimposed on the plateau are seen extended in *Bc*. The steady bias current was +1 and +4 nA in *Aa* and *Ba* respectively.

clonidine-treated cats (triceps surae, $n = 6$, peroneus tertius and brevis, $n = 1$ and plantaris–flexor digitorum longus, $n = 2$).

Figure 4 shows recordings from a motoneurone after I.V. injection of clonidine (0·5 mg/kg). In Fig. 4*Aa* a self-sustained discharge was initiated by a short train of impulses to the contralateral hamstring nerve (cHAM). After several seconds the fast sodium spikes inactivated revealing slow regular oscillations in the membrane

potential (Fig. 4*Ac*). Single or double spikes could be triggered at the peak of the oscillations (Fig. 4*Ab*). From a more depolarized holding potential (Fig. 4*Ba*) the spike-generating mechanism was completely inactivated and a plateau potential (seen more extended in Fig. 4*Bb*) was seen upon which potential oscillations slowly developed (Fig. 4*Bc*). The oscillations disappeared when the plateau potential was terminated either spontaneously (Fig. 4*Aa*) or by an inhibitory synaptic input (Fig. 4*Ba*). Once established the slow oscillations were very regular with a frequency in the range of 7·5–10·5 Hz and an amplitude between 10 and 15 mV. The frequency of the membrane oscillations increased and the amplitude decreased with depolarization of the neurone, while the reverse relation was seen at more hyperpolarized levels. Near to the resting potential the oscillations disappeared. Plateau potentials with superimposed membrane oscillations were also seen with short intracellular pulses (not shown). This and their dependence upon the membrane potential suggest that the rhythmic membrane oscillations are intrinsic properties of the motoneurone.

(4) *The contribution of plateau potentials to the 'late discharge' generated by stimulation of high-threshold afferents*

In the unanaesthetized acute spinal cat intravenous injection of L-DOPA results in profound changes in reflex transmission (Andén *et al* 1966*b*). There is a suppression of a short-latency flexor reflex pathway to motoneurones with a concomitant expression of a late long-lasting flexor reflex on stimulation of high-threshold afferents. Such 'late reflexes' can be seen in flexor and extensor motoneurones following ipsilateral and contralateral high-threshold afferent stimulation respectively (trains of volleys). It was concluded that the 'late reflexes' were transmitted by neural pathways which are inhibited in the normal acute spinal preparation (Jankowska, Jukes, Lund & Lundberg, 1967*a*). Further investigation identified a population of interneurones with response properties strongly implicating them in mediating these 'late long-lasting reflexes' (Jankowska, Jukes, Lund & Lundberg, 1967*b*). However, as bistability induced by L-DOPA (and 5-HTP treatment; see Hounsgaard *et al.* 1988) greatly modifies the input–output relations of motoneurones it is pertinent to ask to what extent do plateau potentials contribute to this long-lasting reflex discharge?

Figure 5*A–C* illustrates a 'late reflex' recorded intracellularly (top traces) from a tibialis anterior (flexor) motoneurone and simultaneously from the corresponding muscle nerve (lower traces) evoked by a train of stimuli to the ipsilateral flexor digitorum longus nerve (50 × threshold). The same reflex was repeated at different intracellular holding potentials. The short-latency discharge was not completely suppressed in this cat as can be seen both in the ENG response and the EPSP in the motoneurone during the stimulus train (Fig. 5*A*). The 'late reflex' is seen as a second 'late' EPSP in the intracellular record (see arrow in Fig. 5*A*) developing within a latency of 100–200 ms from the end of the stimulus train. The time course for the decay of the 'late' EPSP was rather slow (see also Fig. 5*D*) but despite this the duration of the EPSP was shorter than that of the efferent discharge seen in the electroneurogram. With the steady hyperpolarizing current removed (Fig. 5*B*) the 'late reflex' recorded in the motoneurone more closely corresponds with that recorded simultaneously from the muscle nerve. By depolarizing the cell by steady

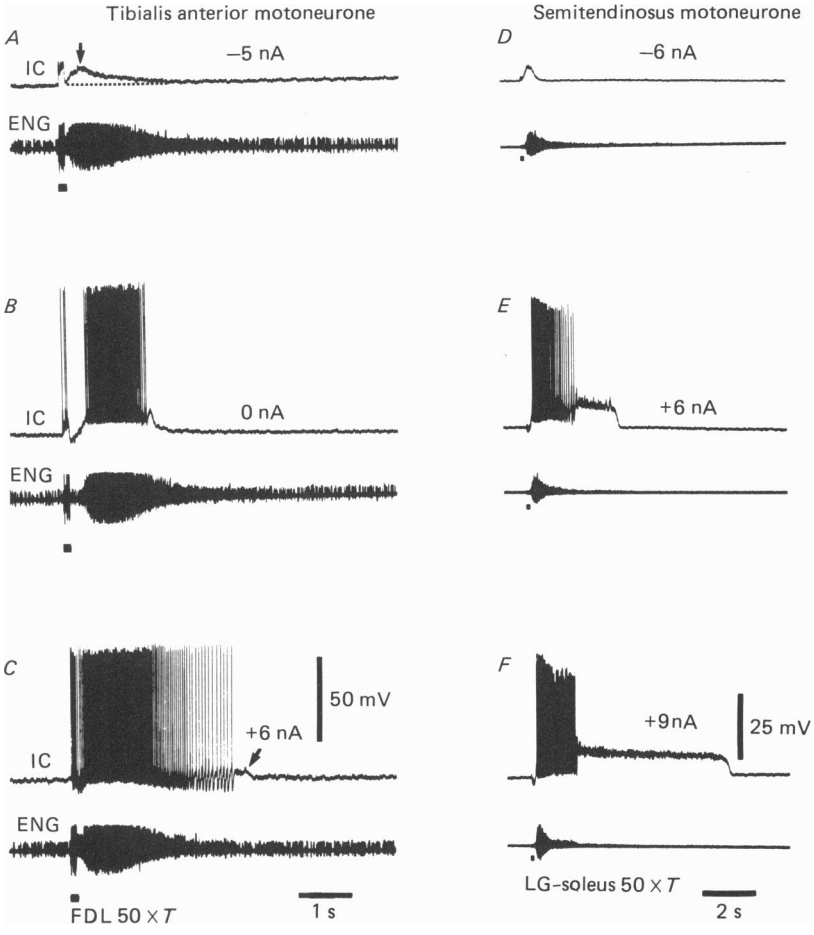

Fig. 5. 'Late discharges' and plateau potentials recorded from a tibialis anterior (*A–C*) and a semitendinosus (*D–F*) motoneurone at different holding potentials in response to high-threshold afferent stimulation. *A–F*, upper traces illustrate intracellular recordings from the motoneurone with corresponding electroneurograms (ENG) shown below each record. The magnitude of current injection to the cell is given to the right of the intracellular records. The 'late discharge' in each example was initiated by a short train of stimuli in ipsilateral high-threshold afferents (flexor digitorum longus nerve, FDL $50 \times T$ in *A–C* and lateral gastrocnemius nerve, LG-soleus $50 \times T$ in *D–F*). The period of stimulation is given by the bar. Nialamide (50 mg/kg) and L-DOPA (100 mg/kg) were given prior to the recordings in *A–C*, while all records were taken after I.V. injection of nialamide (20 mg/kg), L-DOPA (100 mg/kg) and maprotiline (2 mg/kg) in *D–F*.

current injection (Fig. 5*C*) the response of the motoneurone to high-threshold afferent stimulation is greatly facilitated and easily outlasts the main reflex discharge recorded from the periphery. In Fig. 5*C* the cell initially displays a firing pattern that is modulated by the underlying 'late' EPSP (cf. its time course in Fig. 5*A*), but is then followed by a steady low-frequency after-discharge. These observations suggest that the reflex discharge can be considerably more long-lasting than the underlying EPSP by superposition of a plateau potential. In Fig. 5*C* the

arrow indicates the point where the plateau is no longer sustained and spontaneously switches off.

Figure 5*D–F* illustrates intracellular recordings from a semitendinosus motoneurone along with parallel electroneurogram recordings from the semitendinosus motor nerve. In a hyperpolarized state the motoneurone shows a 'late' long-lasting EPSP following high-threshold afferent stimulation (notice that in this example the short-latency effects have been completely suppressed). When the cell was depolarized (Fig. 5*E* and *F*) it is evident that the 'late' EPSP triggers plateau potentials (since the spike-generating mechanism is inactivating, the underlying plateau potential can be directly visualized). The duration of the plateau was very sensitive to the holding potential (compare *E* and *F* of Fig. 5).

Intracellular and parallel electroneurogram recordings were performed in seventeen cats (thirteen treated with L-DOPA or nialamide plus L-DOPA and four cats treated with clonidine) showing well-developed 'late reflexes'. From these experiments a total of twenty-one flexor motoneurones and twenty-two extensor motoneurones displayed behaviour consistent with the results described in Fig. 5.

DISCUSSION

The present series of experiments illustrates that L-DOPA has very similar actions on motoneuronal excitability as 5-HTP (Hounsgaard *et al.* 1988) in acute spinal cats. As descending serotonergic and noradrenergic fibres terminate among, and probably on, motoneurones (Dahlström & Fuxe, 1964, 1965) it is most likely that 5-HTP and L-DOPA act by synthesizing and releasing serotonin and noradrenaline from their respective nerve terminals. However, the striking similarity between the actions of these two monoamine precursors raises a question over the specificity of their actions, i.e. to what extent they share the same receptors or secondary messenger systems. The first section of the Discussion will address this problem. The functional significance of plateau potentials will also be discussed.

The specificity of L-DOPA

Commissiong & Sedgwick (1979) demonstrated that intravenous injection of L-DOPA results in a dose-dependent depletion of serotonin from the lumbar spinal cord in the rat. They proposed that this resulted from the non-selective uptake of L-DOPA, whose subsequent decarboxylation to dopamine within serotonergic terminals leads to a release of stored serotonin (cf. Ng, Chase, Colburn & Kopin, 1970). A substantial release of serotonin following the administration of L-DOPA could possibly explain similarities between the actions of 5-HTP and L-DOPA. However, several experimental findings indicate that the actions of 5-HTP and L-DOPA, despite similarities, are mediated by separate receptor systems.

Bistability in motoneurones following 5-HTP treatment in acute spinal cats is not blocked by the α-receptor blocker phenoxybenzamine but is blocked by methysergide (Crone *et al.* 1988), thus indicating that 5-HTP influences motoneuronal behaviour via serotonergic receptors. This conclusion is further supported by the observation that superfusion of the turtle spinal cord *in vitro* with serotonin induces plateau potentials in motoneurones (Hounsgaard & Kiehn, 1985). In the case of plateau

potentials seen following L-DOPA treatment it would appear that noradrenergic receptors are responsible. This conclusion is based on the following considerations. To induce bistability in 5-HTP-treated cats relatively large doses of the drug are required (Hounsgaard *et al.* 1988). It therefore seems unlikely that corresponding doses of L-DOPA could generate sufficient release of serotonin (via non-specific uptake mechanisms) to explain the presence of bistability in L-DOPA-treated cats. Furthermore, as systemic administration of clonidine (an α-receptor agonist with postsynaptic actions when given in high doses, Andén *et al.* (1970, 1976); Svensson *et al.* (1975)) and topical administration of noradrenaline both induce plateau potentials in motoneurones of acute spinal cats, it would seem reasonable to suggest that these actions are mediated by noradrenergic receptors. It has previously been shown that clonidine mimics most of the effects of L-DOPA on spinal reflex transmission (Forssberg & Grillner, 1973; Andersson & Sjölund, 1978) and that the actions of L-DOPA on spinal reflex transmission are blocked by phenoxybenzamine (and other α-receptor blockers; Andén, Jukes, & Lundberg, 1966*a*). These observations support the concept that L-DOPA acts via the liberation of noradrenaline.

Evidence gained from experiments utilizing ionophoresis or superfusion of serotonin and noradrenaline further indicate that separate receptor systems mediate the action of these monoamines. Serotonin and noradrenaline have been shown to produce a depolarization and an increased excitability of spinal motoneurones both *in vivo* (ionophoresis; McCall & Aghajanian, 1979; Vander-Maelen & Aghajanian, 1980) and *in vitro* (superfusion; Kitazawa, Saito & Ohga, 1985). The serotonin response was blocked by methysergide, whereas the noradrenergic response was blocked by phenoxybenzamine. Furthermore, Kitazawa *et al.* (1985) demonstrated that the depolarization induced by both monoamines persists even in the presence of an extracellular medium with low Ca^{2+} indicating that a second common transmitter is not involved.

Despite the above evidence strongly indicating that the two monoamines act through different receptors the close similarities in their action suggest that they may share secondary messenger and effector mechanisms. Experiments on the isolated turtle spinal cord are presently investigating this possibility (Hounsgaard & Kiehn, 1985, and unpublished observations).

The striking similarities between the action of the two monoamines should not detract from established differences. L-DOPA given to acute spinal rats potentiates a flexor reflex evoked by pinching the toes (see in Austin, Nygren & Fuxe, 1976), while 5-HTP mainly seems to facilitate extensor reflexes seen on tail pinching (see in Nygren, Fuxe, Jonsson & Olson, 1974; Nygren & Olson, 1976). The spontaneous discharge in muscle nerves is regularly increased by 5-HTP and, to a much smaller extent, by L-DOPA (B. A. Conway, H. Hultborn, O. Kiehn & I. Mintz, unpublished observation; cf. Andén *et al.* 1964). In general we observed a differential effect with 5-HTP mainly increasing extensor activity, and L-DOPA increasing flexor activity. This observation correlates well with the experimental finding that 5-HTP reveals bistability mainly in extensor motoneurones (Hounsgaard *et al.* 1988), while flexors as well as extensors may show bistable properties following L-DOPA or clonidine administration.

Rhythmic oscillations of membrane potential

In this series of experiments it was revealed that L-DOPA and clonidine may initiate slow regular pacemaker-like oscillations of the membrane potential. Similar oscillations were never observed in decerebrate or 5-HTP-treated spinal cats (cf. Hounsgaard *et al.* 1988). As the frequency and amplitude of these oscillations are dependent on the holding potential and can be initiated and terminated by intracellular pulses it is most likely that they represent an intrinsic property of motoneurones. These observations demonstrate that in addition to uncovering membrane currents responsible for the generation of plateau potentials α-adrenergic stimulation also activates currents which can result in pacemaker-like potential oscillations. It has been demonstrated that noradrenaline induces a calcium-dependent bursting pacemaker activity in cat sympathetic preganglionic neurones (Yoshimura, Polosa & Nishi, 1987). Noradrenaline has been shown to regulate calcium currents in cardiac muscle cells (Reuter, 1983) and also in some central neurones. To what extent this also applies to motoneurones has to be investigated.

The functional significance of plateau potentials

As discussed in the preceding paper the generation of plateau potentials in motoneurones seems to be an essential mechanism contributing to the stretch reflex in decerebrate cats (Hounsgaard *et al.* 1988). In this paper data are presented which illustrate the contribution of plateau potentials to the late and long-lasting flexor reflexes that are a characteristic of L-DOPA-treated spinal cats (section (4)). The 'late' EPSP triggered plateau potentials in motoneurones and resulted in an augmented reflex output which often outlasted the duration of the underlying EPSP. Despite a contribution from plateau potentials to the long-lasting discharge of 'late' reflexes the underlying EPSP itself is long-lasting (visualized in hyperpolarized motoneurones). It is therefore tempting to speculate that afferent volleys may induce bistable behaviour in the interneurones responsible for generating the long-lasting synaptic excitation. In this context it is interesting to note that interneurones displaying long-lasting discharges and presumably mediating the 'late' flexor reflex have been described (Jankowska *et al.* 1967*b*).

In many invertebrate preparations the function of a neural network has been shown to be dependent on the transmitter-controlled membrane properties of the individual neurones (see Flamm & Harris-Warrick, 1986, for references). The final afferent pattern can be strongly influenced by the induction of plateau potentials not only in neurones generating rhythmic activity but also in the neurones activated by the rhythm generators (e.g. motoneurones; Russel & Hartline, 1982; Dickinson & Nagy, 1983). The capability of nialamide and L-DOPA to initiate fictive locomotion in acute spinal cats (see Grillner, 1981) cannot be directly linked to the bistable properties of motoneurones themselves, but may result from such behaviour in the interneurones responsible for the rhythmicity.

It may turn out that self-sustained discharge and plateau potentials do not occur under natural conditions without support from on-going synaptic excitation. However that would not lessen the importance of plateau potentials; it would only correspond to the experimental finding that the membrane potential is critical for the

expression of bistability. With an appropriate serotonergic and/or noradrenergic innervation the voltage-dependent non-inactivating Ca^{2+}-conductance underlying the plateau (Hounsgaard & Kiehn, 1985) can be expected to provide a large amplification of synaptic excitation. With an amplitude ranging from 5 to 15 mV plateau potentials are likely to be of major importance in providing an increase in the gain of motoneuronal activity. Further experiments on reduced preparations investigating the control of plateau potential threshold and amplitude by monoaminergic innervation are now critical. In this relation it is interesting that motor nuclei, which innervate muscles with pronounced tonic activity have a very dense serotonergic innervation (Kojima, Takeuchi, Kawata & Sano, 1983). From this it seems probable that plateau potentials may be significant in postural control by maintaining tonic activity with a minimum of on-going synaptic excitation.

A 'gain-setting' role has previously been attributed to the descending monoaminergic innervation of motoneurones by several authors (McCall & Aghajanian, 1979; VanderMaelen & Aghajanian, 1982: Kuypers & Huisman, 1982). This notion rests on the observation that ionophoretic application of serotonin or noradrenaline does not excite motoneurones but dramatically potentiates the action of excitatory amino acids (McCall & Aghajanian, 1979; White & Neuman, 1980, 1983; VanderMaelen & Aghajanian, 1982; compare, however, the action of noradrenaline on cat spinal motoneurones described by Engberg & Ryall, 1966, and Engberg & Marshall, 1971). Individual raphe–spinal neurones seem to have widespread projections to cervical, thoracic and lumbosacral segments (Huisman, Kuypers & Verburgh, 1981), and they themselves receive afferent input from mesencephalic, central gray, the lateral hypothalamus and amygdala as do nuclei coeruleus and subcoeruleus (for references see Kuypers & Huisman, 1982; see, however, Aston-Jones, Ennis, Pieribone, Nickell & Shipley (1986), for results indicating a restricted afferent control of nucleus coeruleus). Based on the physiological experiments of Aghajanian and colleagues, as well as on the connections to and from the relevant brain stem nuclei Kuypers & Huisman (1982) proposed that the monoaminergic systems could serve as a special 'gain-setting' component to the motor system 'instrumental in providing motivational drive in the execution of movements'.

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