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A PHARMACOLOGICAL STUDY OF THE ADRENERGIC MECHANISMS INVOLVED IN THE STRETCH REFLEX OF THE DECEREBRATE RAT

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1 The effects of catecholamine precursors, enzyme inhibitors, and monoamine depletors have been studied on the stretch reflex in the decerebrate rat.

2 L-DOPA alone had a biphasic effect, facilitation followed by inhibition and recovery of the reflex.

3 About 10% of the preparations did not develop decerebrate rigidity. These preparations did not have a stretch reflex. Within 1 to 5 min of the injection of L-DOPA in these inactive preparations, a stretch reflex could be elicited.

4 Control experiments indicate that L-DOPA was acting centrally after decarboxylation.

5 DL-dihydroxyphenylserine had an inhibitory effect, without a preceding facilitation.

6 When L-DOPA was preceded by diethyldithiocarbamate (i.v.) or FLA-63 (i.p.), the predominant response was a sustained facilitation, without an accompanying inhibition.

7 It is concluded that in the decerebrate rat, increased stretch reflex activity is associated with increased central levels of dopamine, and decreased stretch reflex activity with increased central levels of noradrenaline. The results are discussed with reference to the possibility that dopamine acts by liberating 5-hydroxytryptamine.

Introduction

L-DOPA (L-3,4-dihydroxyphenylalanine) is now widely used in the treatment of Parkinson's disease, which is associated with low levels of striatal dopamine (Birkmayer & Hornykiewicz, 1961; Gillingham & Donaldson, 1969; Calne, 1970; Yahr & Duvoisin, 1972). High dose levels of DOPA over long periods are frequently accompanied by adverse effects, especially the appearance of involuntary movements. Myoclonic flexion-extension of the limbs is common during L-DOPA therapy, but the mechanism of this effect is not understood (Calne, 1970; see pp. 88-93 and 96-101). One trend in the search for a solution to this problem, has been to study the effects of L-DOPA on spinal reflexes in mammalian preparations (Andén, Jukes & Lundberg, 1966; Grillner, Hongo & Lundberg, 1967; Bergmans & Grillner, 1968; Jurna & Lundberg, 1968; Grillner, 1969; Fedina, Lundberg & Vyklicky, 1971). This work has indicated that although the major beneficial clinical effect of L-DOPA is thought to result primarily from replenishment of striatal dopamine (Barbeau, 1966; Hornykiewicz, 1964, 1966), there may also be considerable direct effects on the lower levels of the motor system.

In the spinal cat, L-DOPA suppresses dynamic gamma motoneurone activity to flexors, and activates static fusimotor activity. A tonic stretch reflex can be elicited from the preparation after L-DOPA, but not before (Grillner, 1969). We have followed up this work on the spinal preparation with a series of experiments on the decerebrate rat. A decerebrate rat was used because a stretch reflex is normally present, which is not dependent on the administration of drugs. We were therefore able to study inhibitory as well as facilitatory effects of drugs.

Our findings are that, after L-DOPA (25 mg/kg i.v.), there is a distinct biphasic change in the stretch reflex; facilitation followed by inhibition and recovery. Evidence is presented to suggest that the facilitation and inhibition are, respectively, associated with increased central levels of dopamine and noradrenaline. Some possible clinical implications of these findings are discussed.

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Methods

Male albino Wistar rats (250-330 g) were used. Tracheal cannulation and mid-collicular decerebration were performed under ether anaesthesia. The left common carotid artery was cannulated and blood pressure was recorded with a Statham P 23 B transducer. Drugs were injected into the femoral artery or vein. The left gastrocnemius-soleus muscle group was used throughout. It was carefully dissected free from the surrounding tissue, leaving nerve and blood supply intact. The Achilles tendon with a slip of bone was cut away from the calcaneum, and the tendon was connected to a Grass FT 10 tension transducer by a stainless steel rod, 6 cm long and 0.6 mm in diameter. The transducer was connected to a Pye-Ling type V 50 MK 1 electromagnetic puller which allowed stretches of varying lengths and rates of displacements to be applied to the muscle. Displacement was measured with a DL/1 D/K Sangamo displacement transducer. Fine silver wire electrodes were used for recording the electromyogram (EMG). In some experiments, the EMG was electronically integrated. The integrator was designed to reset itself, either when the integrator voltage became equal to the power supply voltage, or when the signal to be integrated ceased for a period of 500 milliseconds. One bared electrode tip was placed in the muscle belly near to the motor point, and the other near to the origin of the Achilles tendon. The four parameters—tension, displacement, EMG and blood pressure—were recorded on a Grass Model 7 Polygraph. The wounds were sutured, and the rats were maintained at 35° to 37°C by a thermostatically controlled heating blanket. The femur and tibia were rigidly clamped to prevent movement.

The experimental procedure was as follows: after the preparation had been set up, it was left for at least 30 min to allow the ether anaesthesia to wear off and rigidity to develop. The puller was then adjusted to take up any slack in the muscle, without producing tension at rest. Varying stretches of 1-7 mm at 1.0-2.0 mm/s were then applied to the muscle to find a reasonable stimulating condition, giving a tension of at least 20-30 g/mm. For any one experiment, a standard stimulus, once found was used throughout the entire experiment. This was usually a stretch of 5 mm, applied at 2 mm/s and maintained at the final length for about 4 s; five to eight stretches were applied per minute. When the response to this stimulus was reasonably constant over a period of at least 15 min, the test drug was injected, and any changes in the stretch reflex tension output recorded.

Our interest was in the tonic phase of the stretch reflex. Therefore, the tension values used for plotting graphs were read at least 2 s after the completion of stretch, that is, during the maintained period of stretching. It was unusual for the preparations to exhibit any significant phasic activity at the rates of stretch employed.

Drugs

The drugs used were as follows: L-3,4-dihydroxyphenylalanine (L-DOPA), courtesy of Brocades G.B. Ltd; D-3,4-dihydroxyphenylalanine (D-DOPA), Koch-Light; DL-3,4-dihydroxyphenylalanine (DL-DOPA), Koch-Light; DL-threo-dihydroxyphenylserine (DOPS), Ab Biotec, Sweden. These drugs were dissolved in warm acidified (HCl) 0.9% w/v NaCl solution (saline) to which ascorbate had been added. Bis(4-methyl-1-homopiperazinyl-thiocarbonyl) disulphide (FLA-63) and reserpine base (BDH) were dissolved in warm acidified (CH_3COOH) saline. Sodium diethyldithiocarbamate: *N*-hydroxybenzyl-*N*-methyl, dihydrohydrazinephosphate (NSD-1024): iproniazid phosphate.

The solvents used to dissolve the drugs were injected into suitable preparations and shown to have no effect on the stretch reflex. All drugs were made up to a strength of 10 mg/ml.

Results

The stretch reflex

Figure 1 shows the length/tension relationships of the gastrocnemius-soleus muscles before the injection of drugs. Similar results were obtained in 10 preparations. These results are comparable with those obtained for the soleus muscle of the decerebrate cat by Matthews (1959). The graph shows that after the sciatic nerve had been cut, the passive tension (△) increased due to the passive properties of the muscle. The slope of the linear portion of the total tension curve (○) in this preparation was 83 g/mm.

Active tension (●) was linear over the middle part of its range, but tended to flatten out at higher extensions. This phenomenon is associated with inhibition by Golgi tendon organs in the cat (Matthews, 1959, 1972), and it is assumed that a similar mechanism operates in the rat.

The constancy of the stretch reflex without drugs, once fully developed, was checked in four preparations. A typical result for one such control was a mean tension of 270.0 g (standard deviation of mean = ±40 g), over a period of 1.5 hours. This is an indication that muscle fatigue is not an important factor at the stimulation frequency used

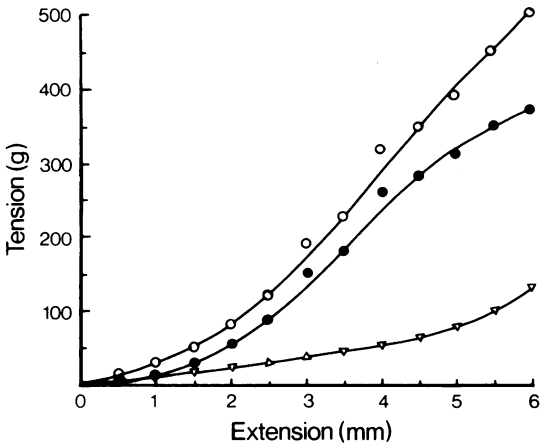

Fig. 1 Tonic stretch reflex in the decerebrate rat. Total (○), active (●) and passive (△) tension developed by gastrocnemius-soleus muscle plotted against extension in 0.5 mm increments. Passive tension was obtained by cutting the sciatic nerve towards the end of the experiment, and stretching the denervated muscle over the same range as the innervated muscle. Active tension ● = ○ — △. The slope of the linear part of the total tension curve is 83 g/mm.

and that significant changes in the stretch reflex do not occur spontaneously during the course of an experiment.

L-DOPA

L-DOPA (25 mg/kg i.v.) produced a consistent biphasic effect on the stretch reflex. An initial facilitation was followed by inhibition and recovery. The time for the completion of the cycle of changes varied widely from 10 to 80 min at the drug dose used. The latency of the response varied from less than 1 to over 5 minutes. Figure 2 illustrates the pattern of the response; total tension is plotted against time. At the peak facilitatory and inhibitory phases of the response, the active tension was 200% and 10%, respectively, of the predrug response. This biphasic response was seen in 12 of the 17 preparations in which this experiment was done. The duration of the facilitatory response was usually 5 to 30 minutes. In most cases, the duration of the inhibitory phase was about twice as long as the facilitatory phase.

During the initial period of facilitation there was sometimes evidence of increased alpha motoneurone activity (Fig. 7B) which might not have been related to the stretch reflex mechanism. However, the EMG voltage during stretch increased with the tension increase and therefore guarantees that the tension increase was due to a neural mechanism and not caused simply by the length-tension characteristics of the muscle, which would have produced a pseudo-stretch reflex (Matthews, 1972). This effect is clearly illustrated in the integrated EMG traces of Figure 3a. During the initial phase of the facilitation the EMG is virtually abolished when the stretch is released.

Three of the remaining five preparations had not developed rigidity or a stretch reflex 45 min after being set up for recording. There was no background spontaneous EMG, and they did not develop active tension during stretch (EMG absent). In all three of these preparations, L-DOPA

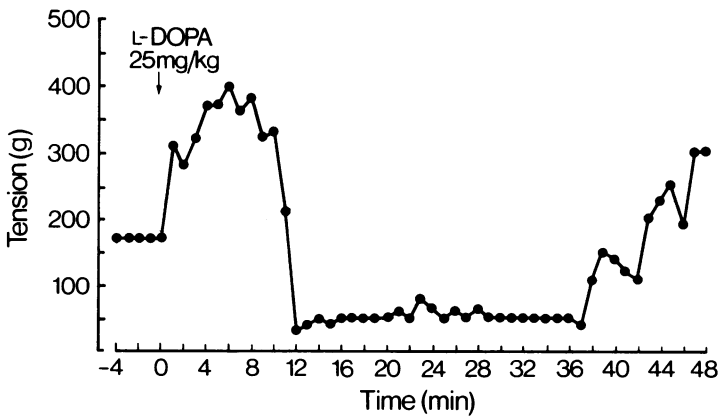

Fig. 2 Biphasic effect of L-DOPA (25 mg/kg i.v.) on the tension developed by the tonic stretch reflex in the decerebrate rat when the muscle was stretched 5 mm at the rate of about six stretches per minute. At peak facilitation and inhibition, active tension had increased to 200% and then decreased to 10%, respectively, of the predrug level. The duration of the inhibitory phase was about twice that of the facilitatory phase. This was the case in 12 out of 17 preparations in which this experiment was done. Recovery occurred with a rebound.

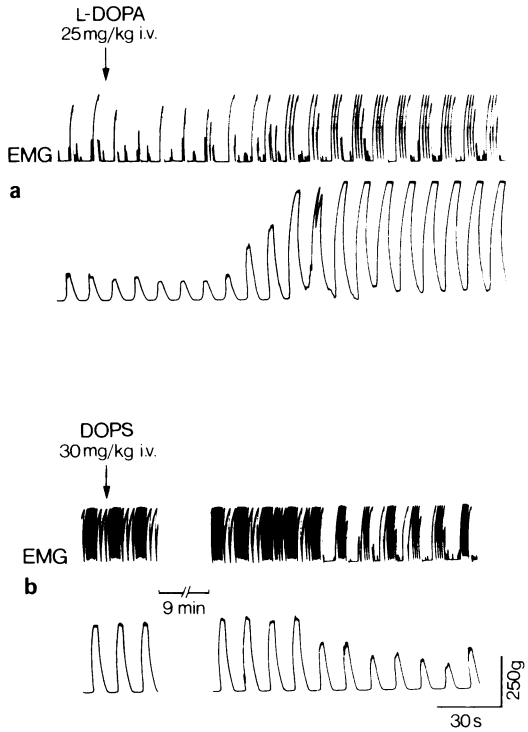

Fig. 3 (a) L-DOPA-induced facilitation of the stretch reflex. The top line represents the integrated EMG and the lower line the tension developed by the muscle in response to stretch. Displacement was constant at 5 mm. Note the pronounced facilitation which occurred with a latency of just under 1 minute. This facilitatory response was maintained for 15 minutes. It is clear that the EMG is abolished between stretches and that increased tension and EMG are directly related. Increase tension is, therefore, not dependent on a generalized activation of the alpha motoneurones. (b) DL-threo-dihydroxyphenylserine (DOPS)-induced inhibition of the stretch reflex. Again, note the direct relationship between EMG intensity and tension. The duration of this inhibitory response was 10 minutes. Note the relatively long latency (12 min) and the absence of a preceding facilitation. Traces a and b are from different preparations.

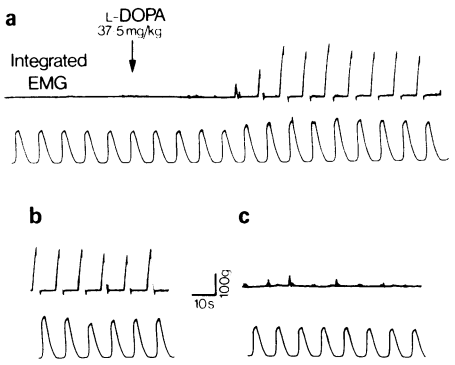

Fig. 4 In a preparation still reflexly inactive 45 min after setting up, L-DOPA (37.5 mg/kg i.v.) induced reflex activity with a latency of about 1 minute. The increase in tension (lower trace) coincided with the appearance of an EMG (integrated), indicating the generation of active tension, (a). (b): 15 min after the initiation of reflex activity, a stretch reflex is still present. (c): 25 min later, the preparation has returned to the inactive state. Passive tension (before L-DOPA, no EMG), was high, and the active tension generated was not great, but quite distinct, as indicated by the EMG.

(37.5 mg/kg i.v.), induced reflex activity within 1 to 5 min of injection. This effect is shown in Fig. 4 where the EMG is integrated. There is no EMG between stretches, therefore the drug has produced some change other than direct activation of the alpha motoneurone.

This effect of L-DOPA on the inactive decerebrate rat is very similar to its effect on the acute spinal cat. In the spinal cat, the static gamma motoneurones are inactive and the stretch reflex is absent. L-DOPA induces static fusimotor activity, and a tonic stretch reflex can then be elicited (Grillner, 1969; Ahlman, Grillner & Udo, 1971). It is possible that L-DOPA has a similar effect on fusimotor neurones in the decerebrate rat.

The other two preparations were both mildly active, with a weak background EMG, and also a weak stretch reflex. However, L-DOPA had no effect on either of them, even after repeated injections.

DL-threo-dihydroxyphenylserine (DOPS)

DOPS is an analogue of L-DOPA. The only structural difference between the two molecules is that DOPS has a hydroxyl group on the β-carbon atom. DOPS is therefore decarboxylated directly to noradrenaline, and dopamine is bypassed on this metabolic pathway. It has been shown that, after DOPS, the central levels of noradrenaline are increased, while those of dopamine are unchanged (Creveling, Daly, Tokuyama & Witkop, 1968; Svensson, 1970).

It was found that a dose of DOPS (25.0-30.0 mg/kg, i.v.) was needed to produce consistent, reproducible changes in the stretch reflex. The drug was tested in eight preparations, of which six had an active stretch reflex. The other two were

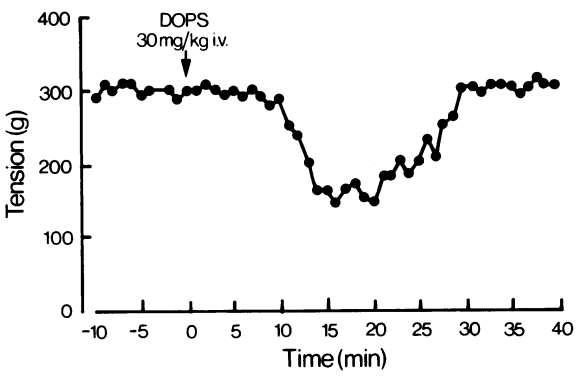

Fig. 5 Effect of DL-threo-dihydroxyphenylserine (DOPS) (30 mg/kg i.v.) on the stretch reflex. There was a distinct inhibition occurring after a latency of 10 minutes. The duration of the inhibition was 20 min, and was followed by recovery. At peak inhibition (20 min after DOPS), the tension was just less than 50% of the predrug response, as measured by total tension values. See Fig. 3b for trace illustrating the DOPS-induced inhibition of the stretch reflex.

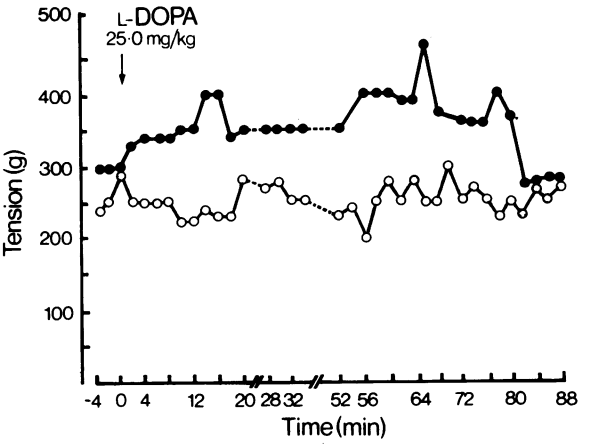

Fig. 6 (•) A sustained facilitation after L-DOPA (25 mg/kg i.v.) in the diethyldithiocarbamate (DDC) pretreated (25 mg/kg i.v. 45 min previously) preparation, without a subsequent inhibition. DDC alone had a general depressant effect in the first 15-20 min; however, a constant response was always obtained before injection of DOPA. Stretch 5 mm. (○) In a control experiment in a preparation of similar sensitivity, an equal volume of the solvent (acidified saline + ascorbate) used for dissolving DOPA was injected, and the fluctuation in tension checked over the same period of time during which the DDC/L-DOPA effect was measured in the test preparation. The mean control value was 270 g (s.d. = ±40 g). Essentially similar results were obtained from FLA-63/L-DOPA preparations. Note the interruption in the time axis.

inactive. DOPS inhibited the stretch reflex in all six active preparations (Figure 5). The inhibition was not preceded by a facilitation in five of these six preparations. In the sixth preparation, a mild facilitation lasting for 7 min preceded the inhibition. The latency of the effect varied from 5 to 12 min, and the duration from 15 to 25 min (Figure 5). Note here, the steady base line preceding the injection of DOPS, and the excellent recovery. The trace from a typical DOPS-induced inhibition is illustrated in Figure 3b. Note the corresponding reduction of both the integrated EMG and tension.

DOPS (up to 45.0 mg/kg, i.v.) was unable to induce stretch reflex activity in the inactive preparations. This negative effect of DOPS contrasts sharply with the activating effect of L-DOPA.

Diethyldithiocarbamate, FLA-63 and L-DOPA

Dopamine-β-hydroxylase is a copper containing protein (Freidman & Kaufman, 1965; Goldstein, Lauber, Blumberg & Peisach, 1965). It was inhibited in these experiments by two agents which act by chelating the copper (Freidman & Kaufman, 1965).

Diethyldithiocarbamate (DDC; 25.0 mg/kg, i.v.) was injected at least 30 min before L-DOPA. The reflex was tested at various times in the interval before giving DOPA. DDC alone appeared to have a generalized depressant effect on the preparation, and tended to cause greater fluctuations in tension within the first 15 to 20 minutes. We always waited until the reflex was again stable before injecting DOPA.

In 16 preparations in which DDC/DOPA was tested, a pronounced long-lasting facilitation without a subsequent inhibition was seen in seven of them (Figure 6). In five, a biphasic effect resembling that seen after L-DOPA alone was observed. In two, there was no change. In two, there was a mild but distinct inhibition.

The high doses of DDC used by some workers were avoided in these experiments (Jurna & Lundberg, 1968). It is therefore possible that, in those preparations in which a biphasic effect was still observed, the enzyme had not been completely inhibited before DOPA was injected. The inhibition seen in the two preparations is difficult to explain on the basis of the hypothesis which we are proposing.

FLA-63 is bis(4-methyl-1-homopiperazinyl-thiocarbamyl)disulphide (Florvall & Corrodi, 1970). DOPA was tested in 11 preparations given FLA-63

(10 mg/kg i.p.) 1 to 4 h beforehand. In five of these, there was a sustained facilitation, without an accompanying inhibition. The pattern of the response was very similar to that seen in the DDC/L-DOPA preparations (Figure 6). The typical DOPA biphasic effect was still seen in two preparations and, in one, DOPA did not have any effect. In the remaining three, the response was inhibitory.

It is concluded that the inhibition of dopamine-β-hydroxylase resulted primarily in facilitation of the stretch reflex after L-DOPA. A sustained facilitation without an accompanying inhibition was never seen in a preparation given L-DOPA alone. In some preparations, the response to L-DOPA remained biphasic, possibly because the dose of DDC or FLA-63 used, was not enough to inhibit the enzyme totally. The inhibition of the stretch reflex seen in the three FLA-63 pretreated preparations is again difficult to reconcile with our hypothesis. (See discussion section.)

Reserpine and L-DOPA

Reserpine pretreatment (0.6-6.0 mg/kg i.p., 16-68 h previously) caused the development of a very extreme extensor rigidity within 10 min after decerebration. This phenomenon did not persist for, at the completion of surgery about 30 min later, the preparations were then only mildly rigid. Blood pressure was severely depressed. There was a discrete firing of single motor units, and a stretch reflex was characteristically absent (Figure 7a).

Jurna & Lundberg (1968) have stated that L-DOPA has no effect on the reserpine-treated spinal cat. They attribute this to the depletion of noradrenaline, the lack of any direct effect of dopamine, and the prevention of noradrenaline synthesis in this preparation. In contrast, we have found that the reserpine-treated decerebrate rat is supersensitive to the effects of L-DOPA; 12.5 mg/kg of L-DOPA in one preparation (Fig. 7b) caused a facilitatory response equivalent to that normally elicited by 25 mg/kg in the non-reserpine-treated preparation. Furthermore, when the dose of L-DOPA was increased incrementally from 6.25 to 37.5 mg/kg a proportionately greater response was produced, and decreased the time needed to reach peak facilitation.

It was not possible to study the inhibitory phase of the response in this preparation since, at the time of the injection of DOPA, a stretch reflex was usually absent (Figure 7b).

Iproniazid and L-DOPA

Because monoamine oxidase (MAO) inhibitors have been stated to increase the effects of DOPA

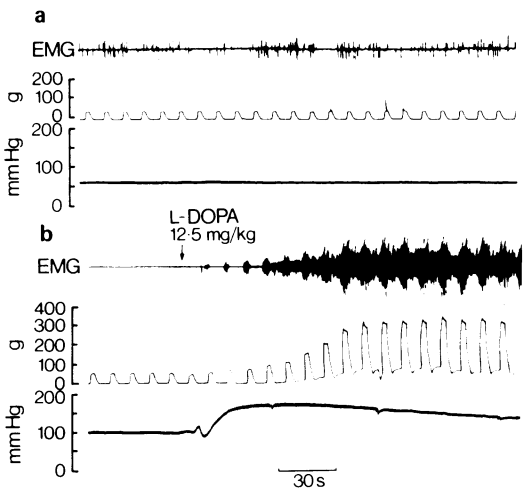

Fig. 7 (a) The effects of reserpine (3.3 mg/kg i.p.) given 20 h before decerebration. There was a discrete firing of single units, as indicated by the EMG, a stretch reflex was absent (middle trace), and the blood pressure (bottom trace) was severely depressed, but very stable. Stretch 5 mm. (b) The effects of 12.5 mg/kg L-DOPA in the decerebrate, reserpine-treated (1.5 mg/kg i.p. 20 h previously) rat. There was a pronounced facilitation of the stretch reflex (middle trace), occurring with a latency of just over 1 minute. Notice the complete absence of any reflex activity during stretch, before DOPA. The facilitation generally persisted from 20-25 minutes. A pronounced pressor effect (bottom trace) was also observed in all reserpine-treated preparations given DOPA. Stretch 5 mm.

by a factor of 10 in the spinal cat (Fedina *et al.*, 1971), it was of interest to test the effects of one such drug in the decerebrate rat. Iproniazid (50 to 100 mg/kg i.p.) was given 16 to 48 h beforehand. A spontaneous background EMG activity was absent in each iproniazid-pretreated preparation. In eight untreated preparations selected at random for comparison, seven had a weak to active background EMG activity, and all eight had a stretch reflex, compared with four out of eight of the iproniazid pretreated preparations.

L-DOPA in the eight iproniazid pretreated animals caused a moderate to strong facilitation in six, but only a mild facilitation in the other two. Three of the six pretreated preparations which gave facilitatory responses were initially inactive, i.e. had no stretch reflex. The average latency for the appearance of the facilitation was about 5 min compared with 2 min in the untreated preparation. Facilitation was also usually brief, but very pronounced. In one preparation, the tension

increased from 90 g before DOPA (passive tension, no EMG) to 450 g within 7 min after DOPA, and only 1 min after the onset of the facilitation. The inhibitory phase of the response, measurable only in the preparations with a stretch reflex before DOPA, was, however, usually very prolonged, lasting for 45 to 75 minutes.

The fact that a spontaneous EMG was absent in all eight pretreated preparations and a stretch reflex present only in half indicated that raised levels of monoamines (most likely noradrenaline), known to result from MAO inhibition, were reducing the excitability of the motoneurones.

L-DOPA and NSD-1024

N-hydroxybenzyl-*N*-methyl, dihydrohydrazine phosphate (NSD-1024) is a general decarboxylase inhibitor, and is known to cross the blood-brain barrier. (Drain, Horlington, LaZare & Poulter, 1962; Porter, Watson, Titus, Totaro & Byer, 1962; Hansson, Fleming & Clark, 1964).

When L-DOPA was injected 1 h after NSD-1024 (50 mg/kg i.v.), there was no significant effect on the stretch reflex. NSD-1024 itself had no significant independent effects on the reflex between the time of its injection and the testing of L-DOPA. We were able to study the effects of L-DOPA for over 3 h after the injection of NSD-1024; during this time repeated injections of L-DOPA had no effect.

D-DOPA, DL-DOPA and L-DOPA

The experiments were done in the reserpine-treated preparation in order to take advantage of the supersensitivity to DOPA shown to be present after decerebration. Twenty-five mg/kg of each drug was used. D-DOPA was inert and did not affect the preparation. DL-DOPA had approximately half of the effect of L-DOPA. This experiment verifies that it is the metabolites of L-DOPA only which are effecting the observed changes in the stretch reflex.

Intra-arterial L-DOPA and DL-threo-dihydroxyphenylserine

A cannula was inserted into the femoral artery until its tip lay at the bifurcation of the abdominal aorta. When 200 to 400 μg of L-DOPA or 100 μg of DOPS was injected into the aorta, it was distributed directly to the left iliac and femoral vascular beds, thereby reaching the left gastrocnemius-soleus muscles. This procedure did not alter the stretch reflex and, therefore, the changes seen after L-DOPA and DOPS i.v. could not be attributed to the effects of the drug acting directly on the muscle spindle.

Discussion

The stretch reflex

A muscle which is actively contracting will develop a tension which is related to its length. The length-tension relationship can be mistaken for a stretch reflex and has been called a pseudo-stretch reflex (Matthews, 1972). In this situation, the activity of the motoneurones is constant and there is no change in the EMG activity of the muscle but an increase in tension is observed on stretching the muscle. To ensure that we were not misled by the pseudo-stretch reflex, we have continuously monitored the EMG signal of the muscle and this appears on the records, either as raw signal (Fig. 7a and b) or integrated with respect to time (Figure 3a and b). Our records show that stretch produces an increase in EMG activity and the tension changes cannot be attributed to the length-tension properties of the muscle.

In some preparations there was an ongoing EMG activity in the muscle upon which the EMG generated by the stretch reflex was superimposed. If, as a result of drug treatment, this ongoing activity changed, then the superimposed stretch reflex would appear to change also. Careful examination of the EMG records however allows one to note any significant change in ongoing muscle activity as opposed to any increase or decrease in that component of the EMG related to stretch. Change in the ongoing muscle activity was observed in some preparations (Fig. 7b) but the change in the stretch reflex was not dependent on its occurrence (Figure 3a).

The question of the site of action of L-DOPA now arises and it is necessary to appreciate that the stretch reflex is not equivalent to the monosynaptic reflex or tendon jerk. The stretch reflex involves the gamma motor system, the Renshaw neurones, segmental and other interneurone circuits all of which have time to modify the discharge of the motoneurone. The decerebrate rat has a relatively intact medulla, pons and cerebellum which could not, of course, be involved in the spinal cat which was used for other studies involving L-DOPA. There are, therefore, a number of sites at which drugs can act to alter the stretch reflex and our experiments give no indication of the precise neural mechanisms involved.

Mechanism of action of L-DOPA

The experiments in which the decarboxylase inhibitor NSD-1024 and D-, DL- and L-forms of DOPA were used show that DOPA itself was inactive and the effects were due to decarboxylated metabolites of L-DOPA. The other experiments indicate that dopamine is associated with

facilitation of the reflex while noradrenaline is associated with depression.

Investigation of the effects of L-DOPA on the reflexes of the spinal cat led to the suggestion that the changes were due to an increased synthesis and release of noradrenaline from reticulospinal noradrenergic terminals (Andén *et al.*, 1966; Bergmans & Grillner, 1968; Andén & Fuxe, 1971; Fedina *et al.*, 1971; Coote & MacLeod, 1972). A modification of this suggestion was that the dopamine formed initially from L-DOPA acted on the noradrenergic terminals causing release of noradrenaline (Jurna & Lundberg, 1968; Fedina *et al.*, 1971; Henning, Rubenson & Trolin, 1972). In either case, noradrenaline was seen as the final mediator of the observed changes. A role for dopamine was not adequately investigated in the experiments on spinal cats, probably because, unlike noradrenaline, it is not present in significant amounts in spinal adrenergic terminals (Dahlström & Fuxe, 1965; Fuxe, Dahlström & Hillarp, 1965). It is clear, however, that these proposed mechanisms cannot explain our findings. We therefore propose that the facilitation of the reflex resulted from increased central levels of dopamine, while the inhibition was associated with increased central levels of noradrenaline.

Action of dopamine

The use of dopamine β-hydroxylase inhibitors, FLA-63 and DDC, allowed us to block the conversion of dopamine to noradrenaline. The resultant long-lasting facilitation of the stretch reflex without a subsequent inhibition, in approximately 50% of the preparations, is strong evidence that high central levels of dopamine mediate the facilitation. The observation of reflex inhibition in five of the 27 preparations in which dopamine β-hydroxylase inhibitors were used is difficult to explain on this hypothesis, but we avoided giving high doses of the drugs and, in these cases, the enzyme may not have been totally inhibited. It is suggested below that the facilitation by dopamine is mediated by indirect mechanisms and it may, therefore, be more labile than the inhibitory response. The finding that DOPS inhibits the reflex without facilitation supports the hypothesis.

The facilitation of the stretch reflex due to increased central levels of dopamine could have arisen as a result of the action of dopamine on the motoneurone or on interneurones in the cord or brainstem, but there are normally no dopamine-containing terminals in the cord and few in the brainstem (Dahlström & Fuxe, 1965). Also, dopamine is usually found to have an inhibitory action on neurones when applied iontophoretically or topically (McLennan, 1961; Hornykiewicz, 1966; Curtis, Duggan & Johnston, 1971; York, 1972), so the facilitation of the stretch reflex would involve the process of disinhibition or disfacilitation of an inhibitory pathway.

Another explanation is possible. Dahlström & Fuxe (1965) and Fuxe *et al.* (1965) have shown that high concentrations of 5-hydroxytryptamine are present in the bulbospinal tract terminals in the vicinity of the motoneurones. It is known that L-DOPA causes 5-hydroxytryptamine to be released from serotoninergic neurones in various parts of the nervous system (Bartholini, DaPrada & Pletscher, 1968; Ng, Chase, Colburn & Kopin, 1970; Everett & Borcherding, 1970; Grabowska, Maj & Mogilnicka, 1971; Okada, Saito, Fujieda & Yamashita, 1972). This release is dependent on the decarboxylation of L-DOPA to dopamine. Dopamine itself releases 5-hydroxytryptamine from brain slices (Ng *et al.*, 1970). 5-Hydroxytryptophan, the precursor of 5-hydroxytryptamine, causes facilitation of the stretch reflex in the spinal cat in a manner similar to L-DOPA (Ahlman *et al.*, 1971; Barasi & Roberts, 1972; Ellaway, Pascoe & Trott, 1973). These workers all note the possible role of the gamma motor system in this facilitation. Further work will be necessary to demonstrate which of these two mechanisms is more important.

The action of noradrenaline

The results of the experiment with dopamine β-hydroxylase inhibitors and DOPS strongly suggest that the inhibition of the reflex is associated with increased central levels of noradrenaline. Noradrenaline is known to inhibit a variety of spinal neurones including the alpha motoneurone (Curtis, 1962; Salmoiraghi & Weight, 1968). We therefore suggest that noradrenaline, formed as a result of increased synthesis and its release from noradrenergic terminals, which use the already available dopamine, mediates the observed inhibition of the stretch reflex. It is possible that the reticulospinal noradrenergic pathway which terminates in the anterior horn plays a major role in this response (Dahlström & Fuxe, 1965; Fuxe *et al.*, 1965). Further support for the inhibitory action of noradrenaline comes from the experiments with iproniazid in which a prolongation of the inhibitory phase of the response was seen after L-DOPA.

Iproniazid and reserpine

If the noradrenaline terminals in the untreated decerebrate preparation are continuously active and releasing noradrenaline, then pretreatment with iproniazid would be expected to reduce the

amount of decerebrate rigidity and activity of the stretch reflex. This was the finding in these experiments. An explanation of the failure of iproniazid to potentiate facilitation of the reflex after L-DOPA could be related to the concept of MAO isoenzymes substrate specificity (Collins, Youdim & Sandler, 1972; Sandler & Youdim, 1972). Iproniazid probably inhibited the isoenzyme which oxidizes noradrenaline, leading to prolongation of the inhibitory phase, but not those which oxidize dopamine or 5-hydroxytryptamine.

The exaggerated extensor rigidity and supersensitivity of the reserpine-treated preparation to L-DOPA is explained as follows. Histochemical evidence suggests that after reserpine-induced monoamine depletion, the 5-hydroxytryptamine concentration at synaptic sites seems not to be as severely depleted as whole tissue levels tend to suggest (Dahlström & Fuxe, 1965). The role of 5-hydroxytryptamine in facilitating the stretch reflex has already been discussed, and in the reserpine-treated preparation it is suggested that dopamine formed from L-DOPA liberates the remaining 5-hydroxytryptamine. Therefore reserpine can be seen as removing an inhibiting agent (noradrenaline) but leaving a facilitatory agent (5-hydroxytryptamine) to act unopposed.

It would now be of interest to repeat this work in other mammalian species to see whether the suggested mechanisms are common to them, as might be expected, since the distribution of monoaminergic pathways from the brainstem is very similar in the rat, guinea-pig, cat and monkey (Battista, Fuxe, Goldstein & Ogawa, 1972). Experiments of a different nature will be needed to determine the exact neural mechanisms for facilitation and depression of the stretch reflex.

Possible significance of this study

The main adverse and dose-limiting effect of L-DOPA in Parkinsonism is the dyskinesias it produces. Involuntary flexion-extension movements occur, which resemble myoclonus (Calne, 1970). It may be that the mechanism responsible for producing this effect is similar to that which produces the exaggerated spinal reflexes in the L-DOPA-treated mammalian preparation. The role of 5-hydroxytryptamine in Parkinson's disease is still obscure (Douglas, 1970; Calne, 1970), but the 5-hydroxytryptamine releasing effect of dopamine, possibly resulting in exaggerated reflexes in mammalian preparations, is worth further consideration in this regard. Dopamine must certainly be formed in the cord and brainstem in high concentrations after the large daily doses used to treat patients. Further, the striatum contains 5-hydroxytryptamine which can be liberated by stimulating the raphé nuclei and its role as a transmitter there must not be overlooked (Holman & Vogt, 1972).

In summary, it is suggested that the findings from this and similar studies (that L-DOPA can produce excitatory effects on the spinal cord of the mammalian preparation) may be of some relevance in attempts to explain some of the adverse effects, especially dyskinesias, accompanying the clinical use of L-DOPA.

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