Selective depression of medium-latency leg and foot muscle responses to stretch by an α_2-agonist in humans

S. Corna, M. Grasso, A. Nardone and M. Schieppati *

*Posture and Movement Laboratory, Division of Physical Therapy and Rehabilitation, Medical Centre of Rehabilitation, 'Clinica del Lavoro' Foundation, IRCCS, I-28010 Veruno (NO) and *Institute of Human Physiology, University of Genoa, I-16132 Genoa, Italy*

1. In standing humans, toe-up rotation of a platform induces a short-latency (SLR) and a medium-latency response (MLR) in both soleus (Sol) and flexor digitorum brevis (FDB) muscles. Toe-down rotation evokes a MLR in the tibialis anterior (TA). The SLR is the counterpart of the monosynaptic stretch reflex, but the origin of the MLR is still debated. By means of tizanidine (an α_2-adrenergic receptor agonist) we tested the hypothesis that the MLR is relayed by group II afferent fibres, since animal data indicate that tizanidine or stimulation of monoaminergic brainstem centres decrease the excitability of spinal interneurones supplied by those fibres. In addition, we compared the effect of the drug on these responses with that induced by stabilization of posture.

2. Eight subjects received tizanidine (150 μg kg^{-1} orally) or placebo, in a single-blind design. Platform rotations were delivered prior to administration and for 3 h afterwards. Both TA- and FDB-MLRs decreased in size, starting from about 1 h after tizanidine administration. Sol-SLR was unaffected. Response latencies were unchanged. Placebo induced no changes in any response. In each subject, the extent of TA-MLR depression induced by holding onto a frame and by tizanidine was superimposable.

3. The selective effect of tizanidine on MLR supports the notion that it is relayed through group II afferent fibres. The similar effects of holding and tizanidine on the response suggests that it is modulated by monoaminergic centres.

It is becoming increasingly clear that, in addition to central output pathways and feedback reflex mechanisms, 'diffuse' neural systems like the monoaminergic systems (Holstege, 1990) play a role in the production of normal motor behaviours. This notion has developed in parallel with our knowledge from animal studies of complex membrane properties of neurones of the brain and spinal cord, and of synaptic transmitters whose physiological actions cannot be readily defined as 'classical' excitatory or inhibitory (Eken, Hultborn & Kiehn, 1989). These actions can be exerted either at a motoneuronal level or at different sites along the pathway from the central terminal of the peripheral afferent fibres to the motoneurones, or both (Bras, Cavallari, Jankowska & McCrea, 1989; Bras, Jankowska, Noga & Skoog, 1990).

The aim of this investigation was to assess in humans the effect of the centrally acting α_2-adrenergic receptor agonist tizanidine, in clinical use as an antispastic agent (Davies, Johnstone, Hill & Quinlan, 1984; Emre, Leslie, Muir, Part, Pokorny & Roberts, 1994), on the stretch reflex induced in the physiological extensor leg and foot muscles by toe-up rotation of a supporting platform. This approach offers a unique opportunity to investigate such effects under conditions in which the muscle response plays a functional postural role. The reflex is composed of two parts, a primary burst corresponding to the almost monosynaptic stretch reflex (short-latency response, SLR) and a secondary burst (medium-latency response, MLR), the origin of which, though still debated, is likely to depend on group II afferent input (Dietz, 1992; Siliotto, Grasso, Nardone & Schieppati, 1995). Therefore, one can test the hypothesis that tizanidine exerts a different effect on these two components. This seems particularly interesting in the light of recent findings in the cat, indicating that the transmission from group II muscle afferents, but not from group I, is depressed by tizanidine and monoamines applied ionophoretically close to the group II recipient lumbar interneurones (Bras *et al.* 1989, 1990), and by stimulation of monoaminergic centres in the brainstem (Noga, Bras & Jankowska, 1992).

Platform toe-down rotation evokes a stretch response in the tibialis anterior muscle, which takes place at about the same latency as that of the MLR of soleus to toe-up rotation. A further aim of this investigation was to see if the tibialis response is affected in a similar way by tizanidine, a finding which would support the notion that the MLRs are mediated by the same type of afferent input and spinal circuit in both flexor and extensor muscles of the leg (Nardone, Corrà & Schieppati, 1990).

In previous papers from this laboratory (Nardone *et al.* 1990; Schieppati & Nardone, 1991), it was shown that stabilization of stance induced by holding onto a stable frame has no effect on the SLR to stretch, but exerts a profound depressive action on the size of the MLR. This phenomenon was attributed to the change in the postural 'set' (see Prochazka, 1989). The time course of this depression (Schieppati, Nardone, Grasso & Siliotto, 1993) is compatible with the time course of the depression induced by locus coeruleus stimulation of potentials evoked in the intermediate zone of the cat spinal cord by volleys in the group II muscle afferent fibres (Noga *et al.* 1992). Therefore, by comparing the effects of tizanidine and stabilization of stance on these responses, one might obtain indirect evidence that the 'set'-connected effect shown in man is mediated by a descending noradrenergic pathway.

METHODS

Eight normal subjects (5 males and 3 females, aged between 24 and 47 years) from the medical staff volunteered for this investigation. Approval for the experiment was granted by the local ethics committee. The subjects stood upright with the eyes open on a platform, which was rotated unpredictably either in foot dorsiflexion (toe-up rotation) or plantarflexion direction (toe-down rotation). The velocity of platform movement was 50 deg s^{-1} and the amplitude was 3 deg. In six subjects showing inconsistent responses in the flexor digitorum brevis to toe-up perturbations under quiet stance, platform rotations were delivered while maintaining a slightly forward-inclined posture. Under these conditions, the constant low level of tonic activity was monitored by the subjects through acoustic and visual feedback of the EMG (Siliotto *et al.* 1995).

Each subject attended two experimental sessions, on separate days. At the beginning of each session, control toe-up and toe-down rotations were delivered, with and without holding onto a stable frame placed in front of the subject. Each subject was then given a single oral dose of either tizanidine (Sirdalud®, 150 μg kg^{-1}; Sandoz Pharma Ltd, Basel, Switzerland), or placebo, in a single-blind fashion. In the following 3 h, the perturbations delivered at a rate of about 0·1 Hz in a pseudorandom fashion (under free stance conditions) were intermingled with periods of rest. In this way, perturbations were clustered in time intervals centred on 35 min before the oral intake of the drug (or placebo) and 10, 55, 100 and 145 min afterwards.

Surface EMG was recorded from the flexor digitorum brevis (FDB), soleus (Sol) and tibialis anterior (TA) through bipolar preamplified electrodes. Signals were filtered (30–500 Hz), post-amplified (× 25), converted analog-to-digital at a sampling rate of 1 kHz and fed into a computer together with the platform position signal. EMG signals were off-line full-wave rectified. The rectified responses obtained during each cluster of perturbations were averaged (*n* = 15–20). The latency of the short- (SLR) and medium-latency responses (MLR) was measured using a cursor on the computer screen (1 sample = 1 ms). Latency was set at the first rising front of the EMG burst, and referred to the onset of platform movement. The end of the responses was set at the time when the activity finally returned to the baseline. The same time window was used to compute the area of the envelope of the response obtained in the control trials and in all the following perturbations.

For the sake of representation and analysis, all mean areas were expressed as a percentage of the mean value of the control responses, and the grand averages of all subjects (and S.E.M.) obtained for each time interval. One-way repeated-measures analysis of variance (ANOVA) was used to assess whether tizanidine or placebo significantly affected the area of the responses. When the result of ANOVA was significant ($P < 0.05$), the Bonferroni multiple-comparisons test was used to assess the time intervals at which the response significantly ($P < 0.05$) changed with respect to the value obtained before drug or placebo administration. Within each time interval, the effect of tizanidine was also compared with that of placebo using Student's paired *t* test. Results are given as means ± S.E.M. unless otherwise stated.

RESULTS

Table 1 shows the latencies and durations for the various responses.

Figure 1 shows three series of responses (averages of rectified EMG) recorded in one subject at various intervals after tizanidine administration. The size of Sol-SLR was unaffected by tizanidine, while Sol-MLR showed a slight decrease in size. In the FDB muscle, the SLR was unaffected by the drug, like in the Sol, whilst the MLR underwent a major reduction in size. The TA-MLR behaved very much like the FDB-MLR. These changes in amplitude were not accompanied by obvious changes in latency.

Figure 2 shows the average changes in the size of the Sol-SLR, FDB-MLR and TA-MLR, obtained at the various time intervals after the intake of tizanidine or placebo, expressed as a percentage of the mean area of the response before administration. All data points suffered from a large scatter, as shown by the error bars. This was due to

Table 1. Latencies and durations for the various responses

Response	Latency (ms)	Duration (ms)
Sol-SLR	37·3 ± 1·1	24·8 ± 1·4
Sol-MLR	68·0 ± 1·7	46·9 ± 7·5
FDB-SLR	53·2 ± 3·2	28·0 ± 1·9
FDB-MLR	90·4 ± 3·1	77·5 ± 9·5
TA-MLR	69·0 ± 2·4	76·1 ± 6·9

major intersubject differences in the amplitude of tizanidine effect (see below). However, the time course of the effect was fairly consistent for the various responses and subjects, so that the MLR of both muscles reached the smallest amplitude in a range from 90 to 145 min.

Analysis of variance showed no difference in the size of responses obtained in the placebo session, but highly significant differences in the MLRs of both FDB and TA after tizanidine. The Sol-SLR showed a minor, nonsignificant increase in size after both placebo and
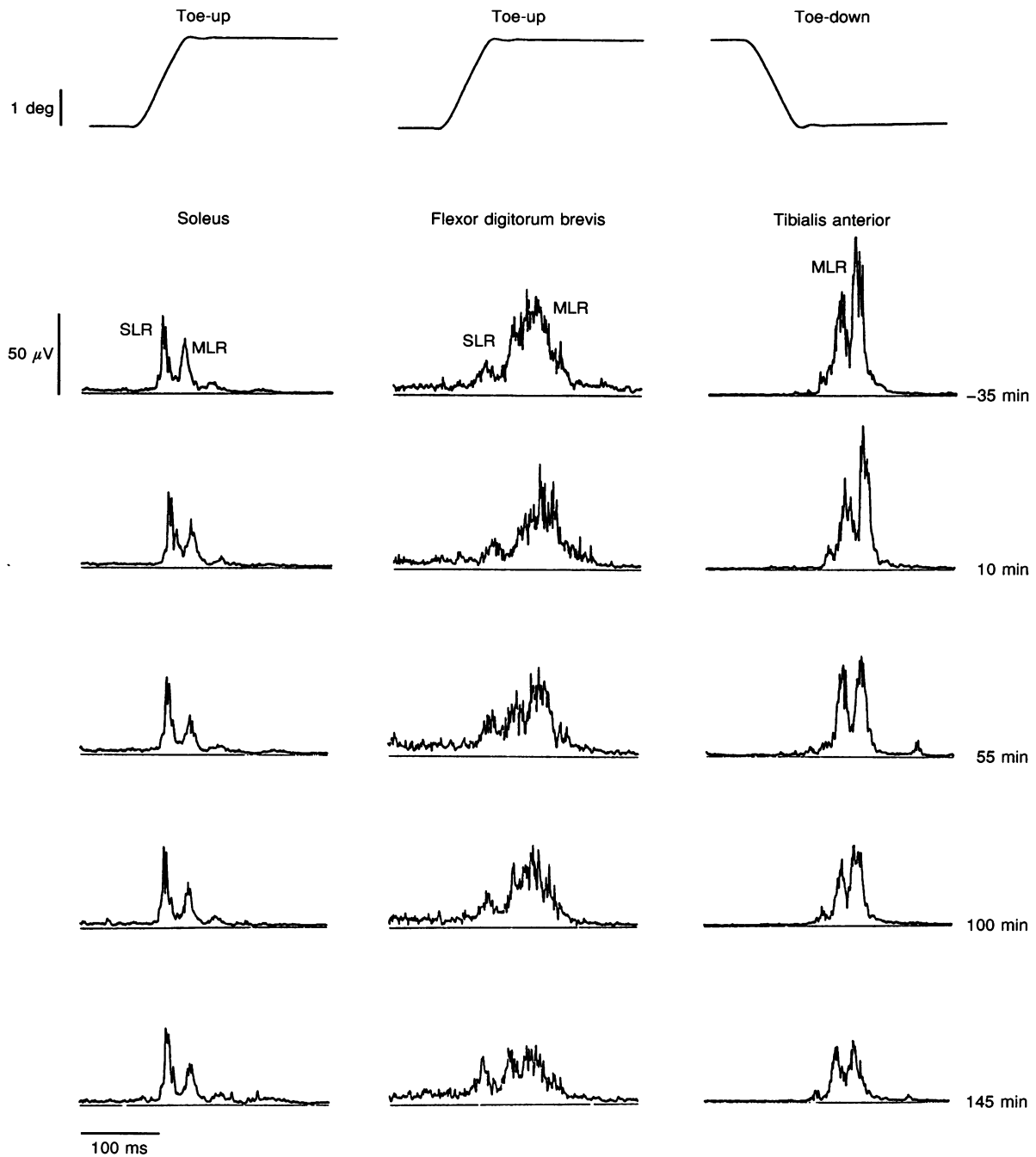
Figure 1. Stretch responses of soleus, flexor digitorum brevis and tibialis anterior muscle at different intervals before and after oral intake of tizanidine

Perturbations were induced by toe-up (left and middle columns) and toe-down platform rotation (right column). Each trace is the average of 15 rectified EMG responses to the postural displacements delivered within a 45 min time interval whose mid-point is indicated to the right of the traces. SLR, short-latency response; MLR, medium-latency response. Horizontal line under each trace represents zero level of the EMG. Already at 55 min after drug intake the MLRs of all muscles have begun to decrease in amplitude, whilst the SLRs are unaffected.

tizanidine administration. The Bonferroni test showed that a significant decrease with respect to the pre-administration value was present for the MLR of both TA and FDB muscles at the intervals centred at 100 and 145 min. However, already at 55 min after tizanidine the MLRs were clearly smaller in both muscles, even if significantly so only in the TA. When the effect of tizanidine on the SLR and MLRs was compared with that of placebo within each time interval, significant differences were present for TA- and FDB-MLRs at the intervals of 55, 100 and 145 min. No significant differences between tizanidine and placebo were found in the case of the Sol-SLR at any interval.

The Sol-MLR and FDB-SLR were small in most subjects, and the least consistent from trial to trial. They were measured in seven and four subjects, respectively. Analysis of variance showed no difference in the size of responses obtained at the various intervals in the placebo session (Sol-MLR, $P = 0.69$; FDB-SLR, $P = 0.64$). There was an indication that tizanidine might have influenced

the Sol-MLR ($F = 1.84$, $P = 0.15$), whilst the FDB-SLR was not affected by the drug ($P = 0.49$).

To see whether tizanidine affected the latency of the MLR, the onset of the TA-MLR was measured on every EMG trace of each subject at both the −35 min interval and at the interval at which the response reached maximal inhibition. The latency of the response after tizanidine administration was just 1.6 ± 0.9 ms shorter than under control conditions, a difference which was not significant.

Before taking tizanidine, all subjects underwent a series of toe-down and toe-up perturbations while holding onto a stable frame. Holding led to a reduction in size of all MLRs, without affecting the SLRs. There were marked differences in the decrease in amplitude of the responses from subject to subject, whilst the latency was unaffected. The correlation between the decreases in amplitude observed under stabilized conditions and tizanidine administration was then investigated, with the hypothesis that a common mechanism subserved both
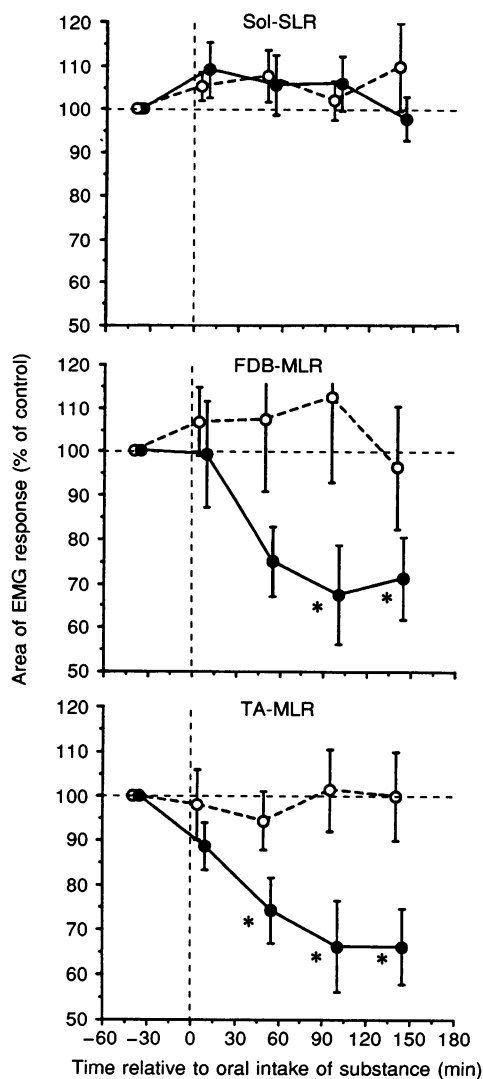
Figure 2. Time course of the average changes in amplitude of SLR and MLRs evoked before and after administration of placebo (○) and tizanidine (●)

Each symbol is the grand mean ± s.e.m. obtained from eight subjects. Response size is expressed as a percentage of the control value obtained before drug intake. Soleus (Sol) SLR is unaffected by either placebo or tizanidine. Flexor digitorum brevis (FDB) MLR is significantly decreased (*$P < 0.05$, Bonferroni test) at the 100 and 145 min time points by tizanidine but not by placebo. Tibialis anterior (TA) MLR is already significantly decreased by tizanidine at 55 min.
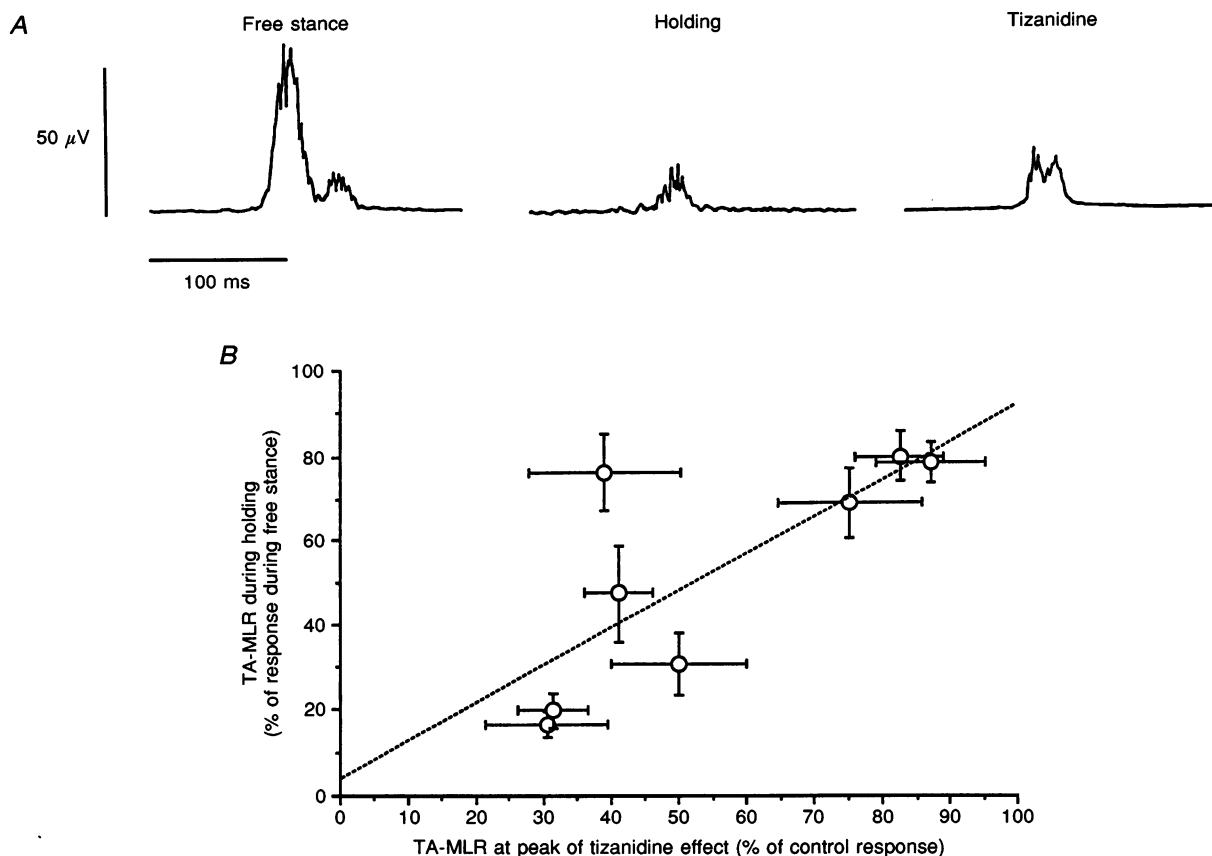
Figure 3. Comparison of the inhibitory effects induced by tizanidine and by stabilized postural 'set'

A, TA-MLR to toe-down rotation of the platform delivered during free stance (left), under holding conditions (middle) and 100 min after tizanidine administration (right). Each trace is the average (*n* = 15) of rectified EMG responses in one subject. Both holding conditions and tizanidine reduce the amplitude of TA-MLR to the same extent. *B*, relationship between the decreases in amplitude of TA-MLR induced by holding (ordinate) and by tizanidine (abscissa) in eight subjects. Symbols are means (± s.e.m.) of 15 responses from each subject. A significant correlation exists between the depression produced by postural stabilization and by tizanidine.

effects. Figure 3*A* shows an example of the effect on the TA-MLR produced by holding conditions and by tizanidine. Both procedures decreased the TA-MLR almost to the same extent with respect to the control value. For each subject, the area of each single response was measured, and its average size obtained under the holding conditions was plotted against the average size obtained at the peak effect of tizanidine (Fig. 3*B*). Tizanidine had the greatest effect on subjects whose response decreased most under the holding conditions, whereas tizanidine reduced the response of only a small percentage in those subjects whose responses were barely affected by holding. The slope of the line drawn through the data points was significantly different from zero, and surprisingly close to the identity ($y = 0.9x + 3.9$; $P < 0.05$; correlation coefficient, $R = 0.76$).

Moderate and transient drowsiness was reported by seven subjects about 1 h following tizanidine administration; four of them also reported a dry mouth. After placebo, drowsiness was reported by three subjects (one of whom was not drowsy with tizanidine). No obvious effects on postural stability during platform rotations were seen.

DISCUSSION

Tizanidine has been shown in studies on primates to depress preferentially polysynaptic (as opposed to monosynaptic) reflex transmission via an α_2-adrenergic mechanism (Davies *et al.* 1984; Corboz, Palmer, Palmeri & Wiesendanger, 1991). In our group of subjects, tizanidine, given at a dose within the recommended daily range (Knutsson, Mårtensson & Gransberg, 1982), markedly

depressed medium-latency responses to stretch, with a time course compatible with that described for clinical effects in patients and for the blood concentrations (Emre *et al.* 1994). This occurred both in the physiological extensor muscle of the foot FDB during dorsiflexing perturbation, and in the ankle flexor TA during plantarflexing perturbation. The MLR in extensor and flexor muscles was depressed to a similar extent. The early, mostly monosynaptic responses, present only in the Sol and FDB muscles, were not depressed by tizanidine.

Since, after placebo administration, no such effects were seen in any subject, either less or more responsive to tizanidine, the response depression cannot be ascribed to a mechanism of adaptation due to the repetition of perturbation *per se*. The amount of response depression varied among subjects a great deal. This may be a sign either of individual differences in the absorption and metabolism of the substance, or of different susceptibility to its pharmacological action (Emre *et al.* 1994), or both. However, in the various subjects, the depressive action of tizanidine was strongly correlated with the depressive effect exerted on the same responses by stabilization of stance (in the absence of tizanidine). On the one hand, this suggests that the inhibitory effect induced by the stabilized postural 'set' is ultimately relayed by the same pathways or mechanisms affected by tizanidine. On the other hand, it would point to a different expression of this physiological mechanism from subject to subject.

The tizanidine effect can be explained on the basis of the effects of monoamines released by coeruleus-spinal pathways on the spinal circuits mentioned in the introduction. Noradrenaline has, in fact, a dual action on the excitability of spinal reflex circuits. It has both a depressive effect relayed by presynaptic inhibitory mechanisms (Riddell, Jankowska & Eide, 1993) on the responses transmitted by group II afferent fibres and relayed through interneurones of the intermediate zone, and an excitatory action on the motoneurones, in turn both direct (Strahlendorf, Strahlendorf, Kingsley, Gintautas & Barnes, 1980; Bras *et al.* 1989) and indirect (disinhibition), mediated by an inhibition of the Renshaw inhibitory interneurones (see Pompeiano, 1989). The excitatory action appears negligible under the present conditions, given the non-significant effect of tizanidine on the SLRs.

On the whole, taking into account the animal findings, the picture that emerges is in keeping with the hypothesis that the primary and secondary responses to stretch are subserved by different afferent pathways and spinal circuits. We favour the notion that, whilst the former response is the counterpart of the monosynaptic reflex, the latter originates in group II spindle afferent fibres (Schieppati, Nardone & Corna, 1995). These findings would also suggest a rationale for the clinical use of α_2-agonist substances as muscle relaxants in humans.

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