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Mechanographic analysis of muscle rigidity after morphine and haloperidol: a new methodological approach*

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Summary. The new method described in this study was based on consecutive repeated measurements of the resistance of flexor and extensor muscles of the hind foot of the rat to forced flexions and extensions of the foot. Locomotor movements of the rat were restrained with a metaplex box which had a slot for the hind limb. The control muscle tone measured by this method was constant for more than 2 h, and amounted to approx. 25 g for flexor muscles, and approx. 45 g for extensors. Morphine (2.5, 5, 10, 20 mg/kg) enhanced dose-dependently the resistance of flexor muscles up to approx. 45 g, 70 g, 100 g and 140 g, respectively, and the resistance of extensors of the paw up to approx. 100 g, 140 g, 180 g and 240 g, respectively. Haloperidol (5 and 10 mg/kg) enhanced dose-dependently the resistance of flexor muscles up to approx. 45 g and 70 g, respectively, and that of extensors of the foot up to approx. 75 g and 120 g, respectively. Morphine rigidity, measured as resistance of respective muscles to forced movements, was almost completely inhibited by a consecutive injection of 0.2 mg/kg of naloxone. The new method seems to have considerable advantages in comparison with electromyographical (EMG) or other kinds of mechanographical measurements of the muscle tone.

Key words: Muscle rigidity – Mechanographic studies – Morphine – Haloperidol

Introduction

Muscle rigidity is a clinical symptom (e.g. Parkinson's disease) not easy to assess objectively. This symptom appears as a side-effect in the course of treatment with neuroleptics and therefore requires a repeated, accurate evaluation (Byck 1975; Hornykiewicz 1975).

The assessment of muscle rigidity is carried out in both man and animals subjectively, or by objective methods. Changes in the muscle tone in response to the limb bending are estimated subjectively by palpation. Objective methods are different variants of electromyographic (Wand et al. 1973; De Ryck and Teitelbaum 1983) and mechanographic (Johnels et al. 1978; Dickinson and Slater 1982; Dickinson et al. 1982) techniques. The latter ones are erratic, technically not easily performed and seem problematic in their interpretation; moreover, they usually do not delimit the contribution of various muscle groups. In this situation it happens that the same compound is evaluated in a different manner, dependent upon the dose and method of assessment; e.g. the neuroleptic drug haloperidol does not affect muscle tone according to some authors (Dickinson et al. 1982 – a mechanographic method; Winkler et al. 1982 – EMG), induces a very specific type of muscle rigidity according to others (De Ryck and Teitelbaum 1983 – EMG), or has a muscle-relaxant effect in still another author's opinion (Anderson 1985).

Since the methods used in those studies yielded divergent results, and since in the clinic the usual treatment with neuroleptics can induce muscle rigidity, it was essential to elaborate a new method which would detect this symptom in animals.

This paper presents a method which detects the haloperidol-induced rigidity in rats and, furthermore, permits evaluation of changes in the muscle tone and resistance of precisely determined groups of antagonistic muscles of the rat's paw. The measurement was conducted by recording successively the resistance of flexors and extensors which counteracted the mechanically forced straightening and bending of the paw in the ankle joint. Morphine was used as a model substance, being generally accepted to evoke muscle rigidity; its action was compared with that of the typical neuroleptic haloperidol.

Methods

The experiment were carried out on conscious, naive male Wistar rats weighing 220–260 g.

Changes in the muscle tone were induced by single subcutaneous injection of morphine (morphinum hydrochloricum, Polfa, Warszawa, Poland, ampoules of 1 ml = 10 mg) in doses of 2.5, 5.0, 10.0 and 20.0 mg/kg (referred to salt), and haloperidol (Gedeon Richter, Budapest, Hungary, ampoules of 1 ml = 5 mg) in doses of 5 and 10 mg/kg (referred to base). Control animals were injected subcutaneously with physiological saline. Some rats were treated intraperitoneally with 0.2 mg/kg of naloxone (naloxone hydrochloride, Narcan, Winthrop Labs, ampoules of 1 ml = 0.4 mg) (referred to salt) 1 h after administration of 20 mg/kg of morphine.

Automatic mechanographic assessment of muscle resistance. A mechanographic assessment was carried out recording

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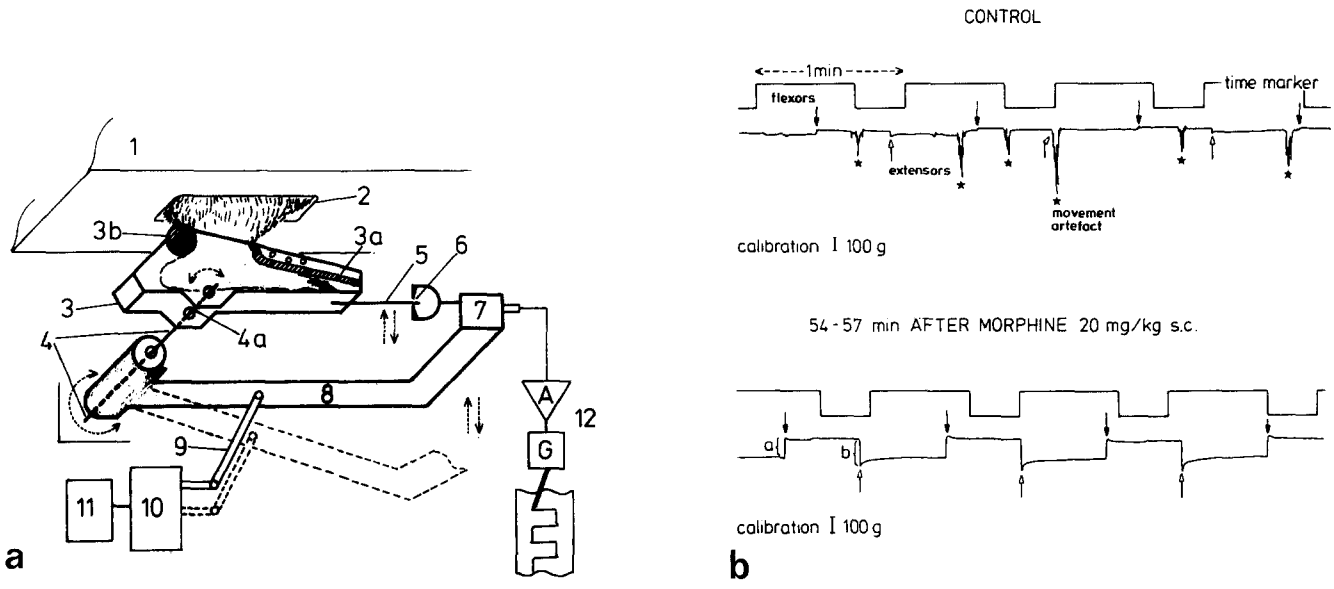

Fig. 1. a A schematic drawing of the device for an automatic mechanographic assessment of muscle tone; *1* a metaplex cage limiting the locomotor movement of the rat; *2* an opening in the bottom of the metaplex cage for the hind limb of the rat; *3* a metaplex box for the foot of the rat – the "shoe"; *3a* a displaceable lid of the shoe; *3b* a metaplex bar placed behind the Achilles tendon for fixing the foot; *4* a common rotation axis for both the arm "8" and the shoe "3"; *4a* openings in the base of the shoe for the axis "4"; *5* a steel rod, sunk firmly on one side in the base of the shoe and introduced on the other side into the measurement opening of the force sensor; *6* a measurement opening of the force sensor; *7* a force sensor, Grass Force-Displacement Transducer FT03B; *8* an arm moving the force sensor fixed firmly on it; *9* an arm moving the arm "8"; *10* a power transmission system; *11* an electronic system controlling the power transmission system; *12A* an amplifier, Grass polygraph, 78B; *12G* a galvanometer, Grass polygraph; **b** fragments of original mechanographic recordings; the upper part – a control recording after subcutaneous injection of physiological saline; the lower part – a recording obtained 54–57 min after 20 mg/kg of morphine; *filled arrows* dynamic tension of flexors of the foot which developed in response to forced straightening (extension) of the paw; *open arrows* dynamic tension of extensors of the foot which developed in response to forced bending (flexion) of the paw; asterisks – movement artefacts (spontaneous movements of the paw); a and b – values of the dynamic muscle tone (peak resistance due to reflex activation by stretching) of flexors and extensors, respectively, used for calculation of means and SEM; *g*, grams.

successively the resistance of flexors and extensors which counteracted the forced straightening and bending of the foot in the ankle joint.

A schematic drawing of the functioning of a device for the mechanographic measurement of the muscle resistance is shown in Fig. 1 a. In order to carry out the measurement a conscious rat was placed in a special well-ventilated metaplex cage (1) (Fig. 1 a) and its hind limb foot, protruding from a special opening (2) in the bottom of the cage, was slipped into an appropriately matched box (3) – the "shoe" – also made of metaplex. The construction of the shoe permitted a comfortable horizontal positioning of the foot in a manner adapted in an optimal way to its physiological resting position. A special blockade limited the movements of the foot in the shoe to a necessary minimum. The blockade of the foot was double and consisted of: 1) an appropriately modelled displaceable lid of the box (3a), whose position could be adapted to the size of the foot, and 2) a metaplex rod (3b) inserted into appropriate openings back to the Achilles tendon which prevented the pulling out and moving back of the foot in the shoe. There was a rigid steel rod (5) sunk into the base of the shoe. The other side of the rod (5) was inserted into a measurement opening (6) of the force sensor (Grass Force-Displacement Transducer FT03B) (7). In this way each change in the pressure exerted by the foot on the shoe (due to reflex activation by stretch) was transmitted immediately via the steel rod (5) on the force transducer (7) and registered by the Grass polygraph (model 78B) (12). The axis (4), allowing rotations of the metaplex shoe (3), was placed exactly under the ankle joint (4a). The localization of the axis (4) stabilized the position of the foot and allowed the measurement of the response restricted to flexors or extensors of the foot, and thus eliminated the influence of other muscles of the limb.

The rat's foot was forced to move in the following manner: an electronic control system (11), connected to the power transmission system (10), controlled the time of straightening or bending of the foot ($t = 333$ ms) and a reproducible quantity of the foot movement. The power transmission system (10) set the arm (9) in motion, which, in turn, induced a rotary motion of the arm (8) around the axis (4). The arm (8) shifted the force sensor (7) (lowered or raised by 10°) which was firmly attached to it. The force sensor (7) forced the movements of the show (3) via a steel rod (5). In this way the rat's foot was forced to move. A response to the forced movements of the foot was the resistance of respective muscles, due to the reflex activation by stretching extensors at bending (raising) the foot, and flexors at its straightening (lowering). At intervals between successive bending and straightening movements the paw was reposed for 30 s in a horizontal position, i.e. at bending, and for 35 s in a position of partial (10°) straightening. The movements of straightening and bending the foot were repeated regularly every 65 s throughout the experiment. The calibration of the apparatus was carried out each time after termination of the experiment and after taking the rat out

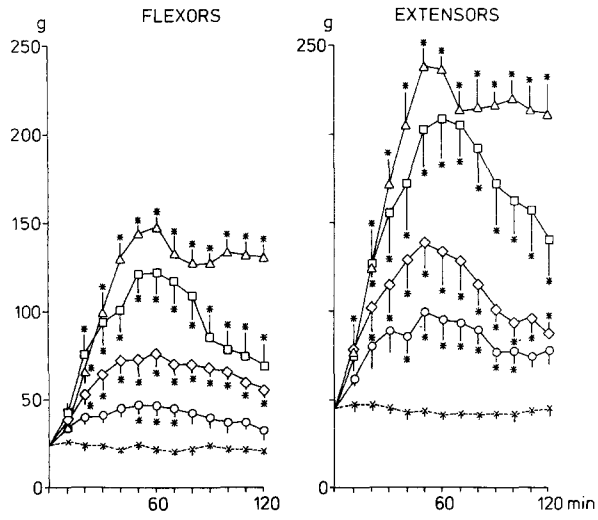

Fig. 2. The effect of morphine on the muscle tone of flexors and extensors of the rat hind paw; each point represents a mean of $9 \times n$ individual values of the dynamic muscle tone, vertical bars represent $\pm$ SEM (+ or −), ordinate − force in grams (g), abscissa − time in minutes; *x* control solvent injection ($n = 6$), *open circles* 2.5 mg/kg of morphine, sc ($n = 6$), *open diamonds* 5 mg/kg of morphine ($n = 6$, *open squares* 10 mg/kg of morphine ($n = 8$), *open triangles* 20 mg/kg of morphine ($n = 7-11$); *asterisk* difference significant at least at $P < 0.05$ to the control group

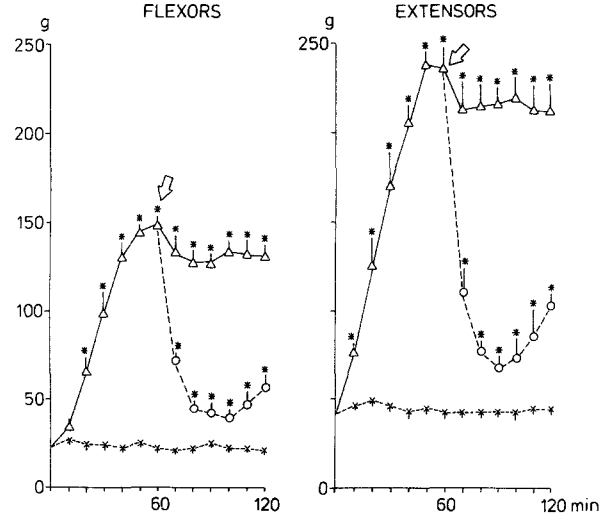

Fig. 3. The influence of naloxone on the morphine-increased dynamic muscle tone of the rat hind paw; *x* control solvent injection ($n = 6$), *open triangles* mean values of the increased dynamic muscle tone induced by 20 mg/kg of morphine, sc ($n = 7-11$), *open circles* the effect of 0.2 mg/kg of naloxone, sc ($n = 4$), on the morphine-increased dynamic muscle tone [each open circle represents a mean value which is significantly different at $P < 0.05$ from the respective value obtained after injection of morphine alone (*open triangles*)]; *large open arrow* injection of 0.2 mg/kg of naloxone; *asterisk* − difference significant at least at $P < 0.05$ to the control group; for further details see Fig. 2

of the cage, having hung a weight of 100 g at a fixed point of the steel rod (5). The proper experiment was preceded by a 45-min period of adapting the animal to the experimental protocol, which reduced to a considerable degree movement artefacts.

The mean resistance values of flexors and extensors of the foot were calculated from 10-minute recording periods after elimination of artefacts resulting from spontaneous limb movements (Fig. 1 b). The value of the dynamic muscle tone (the peak resistance due to the reflex activation by stretching) of the respective muscle group is indicated by the largest value (highest point of the curve) of muscle tension which developed during and at the end of stretching in response to a passive forced movement of the foot (333 ms) (Fig. 1 b). The static or tonic value of reflex tension of the muscles is indicated by the values of muscle tension which developed during the sustained stretching (which followed by 30 s or 35 s the dynamic phase of stretching). The numerical values of the tonic reflex tension are not shown in the figures.

The statistical analysis of differences was carried out by the one-way ANOVA and Duncan test.

Results

The recording of the resistance (dynamic muscle tone due to the reflex activation by stretching) of flexors and extensors of the rat's paw for a period of 4 h demonstrated that the same control values in response to a forced bending or straightening of the paw were maintained throughout the experiment. Those values were approximately 25 g for flexors and 45 g for extensors. Like in the case of the 2-h control measurement presented in Fig. 2, differences between the respective means of 10-min measurement periods did not exceed a few grams. The resistance of the paw extensors, both during the control experiment and after administration of morphine or haloperidol, was almost twice as high as that of the flexors (Figs. 2, 3, 4). The increases in resistance in both groups of the paw muscles were directly proportional to the doses of both drugs (Figs. 2 and 4). The method used to measure the muscle resistance permitted a detection of a stimulating effect of morphine on the resistance already at doses of 2.5 and 5 mg/kg (Fig. 2). A low dose of naloxone (0.2 mg/kg), administered 60 min after morphine, reduced the morphine-induced increase in the muscle tone of both flexors and extensors of the paw almost to control values (Fig. 3). Haloperidol in doses of 5 and 10 mg/kg increased significantly and dose-dependently the muscle resistance of flexors and extensors of the paw (Fig. 4). The increase in muscle resistance after haloperidol, 10 mg/kg, resembled the effect evoked by morphine, 5 mg/kg.

Discussion

The paper presents a new method of the mechanographic assessment of the muscle tone of flexors and extensors of the hind paw of the conscious rat. The resistance developed in response to a forced straightening and bending of the paw, and was due to the reflex activation of the respective muscles by stretching. The dynamic value of the muscle tone (i.e. the peak resistance of the respective muscle group) is expressed by the largest value of muscle tension which developed in response to the passive forced movement of the foot, i.e. during and at the end of stretching (Fig. 1 b). The static or tonic value of the reflex tension of muscles is indicated by the values of the muscle tension which developed during the sustained stretching (which followed by 30 s or 35 s the dynamic phase of stretching).

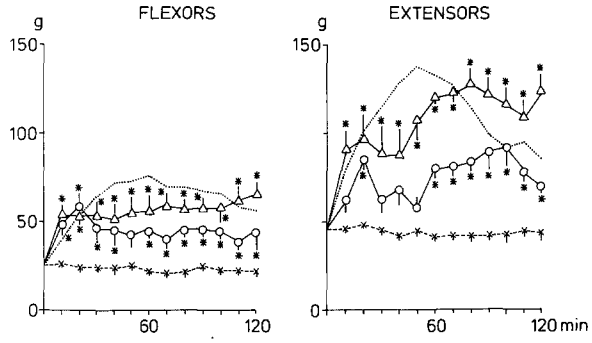

Fig. 4. The effect of haloperidol on the dynamic muscle tone of flexors and extensors of the rat hind paw: *x* control solvent injections ($n = 6$), *open circles* 5 mg/kg s of haloperidol ($n = 5$); *open triangles* 10 mg/kg s of haloperidol ($n = 7$); *pointed line* the effects of morphine, 5 mg/kg sc, on the muscle tone; Note: the effect of 10 mg/kg of haloperidol is almost the same as that of 5 mg/kg of morphine (dotted lines); for further details see Fig. 2.

The method is a laboratory model of the simplest clinical technique of measuring muscle rigidity – a subjective assessment of resistance of the patient's, hand bent in the elbow joint by an examining physician. However, in our method the subjective assessment by the examining physician was substituted by an objective measurement of the muscle tension, which permitted a precise determination in grams. Moreover, advantages of the present method are: 1. directness of the assessment of changes in the muscle resistance and tone, in contrast to electromyographic methods which measure it indirectly through the muscle electrical activity (Wand et al. 1973; De Ryck and Teitelbaum 1983), 2. assessment of the dynamic value of the muscle tone, i.e. of the resistance which develops in response to the forced movement, 3. limitation of the measurement to strictly determined groups of muscles: the flexors and extensors of the paw, 4. an almost concurrent measurement of the resistance and tone of antagonistic muscles – flexors and extensors – of the paw in the same animal, 5. stabilization of the measurement due to maintaining the paw in an identical position throughout the assessment period, 6. harmlessness to the examined animal and, therefore, a possibility to repeat the measurements a number of times in the same animal.

The method described above is very sensitive and reproducible. Using this method we detected a significant increase in muscle tone after doses of morphine (2.5 and 5 mg/kg) 4 times lower than those necessary to evoke the rigidity measured by electromyographic methods (10 mg/kg) (Wand et al. 1973; Ossowska et al. 1986). The reproducibility of the method is indicated by a strikingly small scattering of results (Figs. 2, 3 and 4), which permits limitation of the number of animals necessary for the experiment and, therefore, diminution of the expenditure of work and time needed to carry it out. Recently this method has become automatized to a considerable degree, which hastens calculations.

It was interesting to find that haloperidol increases the muscle tone. As has been mentioned in the Introduction, there are diverse opinions on this subject. It has been suggested that haloperidol has no action on the muscle tone in animals (Dickinson et al., 1982; Winkler et al., 1982), or even exerts a muscle relaxant effect (Anderson 1985), whereas it is inferred from clinical observations that butyrophenones induce particularly potent extrapyramidal effects in man (Byck 1975; Hornykiewicz 1975). Only a technically complex method using many permanently implanted muscle electrodes and an electromyographic recording in freely moving animals, applied recently by De Ryck and Teitelbaum (1983), permitted detection of haloperidol-induced increase in the muscle tone in response to a change in the animal's position. The latter, most interesting method of great cognitive value is, nevertheless, technically too difficult and too expensive to be used for routine screening studies; moreover, it does not permit a direct evaluation of changes in the muscle tone in a manner comparable with the clinical test. The method presented in this paper avoids both these obstacles; at the same time it confirms the main conclusion of De Ryck and Teitelbaum (1983), as well as the clinical opinions which indicate that haloperidol used at high doses enhances the muscle tone.

Dickinson and Slater (1982), Dickinson et al. (1982) studied changes in the muscle tone by a method similar to ours. The inconvenience of that method lies in the fact that it measures the tone of muscle groups that are difficult to determine; moreover, it is difficult to intepret the results obtained by the experimental procedure in which the animal's limb hangs freely from an opening in the bottom of the cage, not fulfilling its basic function, i.e. the maintenance of the rat's body in a position appropriate for this species. During elaboration of our method we tried to fix the rats' paw in a position in which – under natural conditions – the paw sustains the body of the rat. That procedure permitted us to increase approximately tenfold the sensitivity of assessment. Dickinson et al. (1982) obtained an increase of 20 g in the muscle tone after administration of morphine, 12 mg/kg, while our method allowed us to obtain an increase of approximately 160 g in extensor muscles when the same drug was used in a dose of 10 mg/kg.

It is also noteworthy that the muscle tone of the paw extensors was considerably higher than that of the flexors, which can be easily explained by an antigravitational character of these muscles. This fact adds to the value of our method which measures the muscle tone under more physiological conditions. Further studies onto the method described in the present paper, conducted currently, suggest that after some modifications it can be used for detecting the muscle relaxant action of a given compound, as it is already more sensitive than the method of Dickinson et al. (1982) in discovering changes in the muscle tension.

Our study presents a new method of assessing the muscle tone of flexors and extensors of the rat paw, which in many respects seems to be more advantageous than the techniques used so far. It seems suitable not only for a wide range of pharmacological screening studies, but may also advantageously complement the more complicated electromyographic methods which may be used mainly to study the intrinsic mechanisms of rigidity.

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