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Quantitative grip strength assessment as a means of evaluating muscle relaxation in mice

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Abstract. The effects of various centrally acting drugs and some peripherally acting agents on the forelimb grip strength of CD-1 mice were explored. Forelimb grip strength was assessed by use of a strain gauge to measure the lateral pull force, in grams, exerted by mice as an index of muscle relaxation. The muscle relaxants, diazepam, midazolam, baclofen, methocarbamol, dantrolene sodium and the neuromuscular blocking agents, succinylcholine and pancuronium bromide, dose-dependently reduced forelimb grip strength. 2-Amino-7-phosphonoheptanoic acid (AP7), which has also been shown to have muscle relaxant effects, also reduced grip strength. Pentobarbital, ethanol, phencyclidine, ketamine and chlorpromazine reduced grip strength at doses which produced behavioral impairments. Lithium chloride, a toxic compound used to induce taste aversions, and clonidine, at doses which affect blood pressure, body temperature and locomotor activity, did not affect grip strength. In addition, stimulant doses of amphetamine and caffeine, but not of morphine, increased grip strength in a dose-dependent manner. These results extend previous findings and suggest that this forelimb grip strength procedure may be a useful screening test for the identification of the potential muscle relaxant properties of drugs.

Key words: Grip strength – Muscle relaxation – Skeletal muscle relaxants – Sedatives – Stimulants

The quantitative assessment of grip strength in rodents has been used for several different purposes. Grip strength is commonly used as an indicator of neurotoxicity in that grip strength evaluation comprises one test in a battery of tests for assessing the neuromotor effects of chronic exposure to neurotoxicants such as hexane (Howd et al. 1983), acrylamide (Gerber and O'Shaughnessy 1986) and soman (Haggerty et al. 1986). In addition, grip strength has been used as part of a test battery to assess biological age in mice (Ingram and Reynolds 1986).

Acute impairment in grip strength by centrally-acting drugs has been used to infer muscle relaxation. Using a variety of instruments and methods to assess grip strength, muscle relaxants such as diazepam (Van Reizen and Boersma 1969; Soubrie and Simon 1978; Simiand et al. 1989), tetrazepam (Simiand et al. 1989), baclofen (Simiand et al. 1989), orphenadrine (Van Reizen and Boersma 1969), mephenesin (Simiand et al. 1989), chlormezanone (Van Reizen and Boersma 1969; Simiand et al. 1989), chlorzoxazone (Leszkovszky and Tardos 1970) and meprobamate (Van Reizen and Boersma 1969), and major tranquilizers such as chlorpromazine (Van Reizen and Boersma 1969) have been shown to reduce grip strength in mice. Phenobarbital and chlordiazepoxide have been shown to reduce grip strength in rats (Meyer et al. 1979).

Little is known about the effects of other types of centrally acting drugs on grip strength. In addition, little is known about the sensitivity of the grip strength measure to peripherally acting agents. The current study examined the effects of a variety of drugs on forelimb grip strength in order to determine whether forelimb grip strength is differentially reduced by drugs known to possess muscle relaxant activity. Toward this end, test drugs were selected to represent; 1) known muscle relaxants acting through various mechanisms and at different sites, and 2) drugs of various pharmacological classes that are believed to possess little or no muscle relaxant effects, but which have various effects on behavior. If forelimb grip strength were found to be differentially reduced by known muscle relaxants, it would suggest that assessment of forelimb grip strength could be a useful screening procedure to identify potential muscle relaxant activity.

Materials and methods

Subjects. Experimentally naive male CD-1 mice (Charles River Laboratories, Portage, MI) approximately 30–40 days of age, weighing 20–30 g were housed seven per cage in a temperature and humidity controlled vivarium on a 12 h light/12 h dark cycle. Testing was conducted during the light portion of the cycle. Food and water were available ad libitum. For studies in which drugs (i.e., ethanol and dantrolene Na) were administered orally, the mice were fasted overnight prior to testing.

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Apparatus. The grip strength apparatus was similar to that described by Meyer et al. (1979). Briefly, a Chatillon (Greensboro, NC) model DPP-0.5 kg push/pull gauge (calibrated in 5 g units) was mounted horizontally 2 cm above a platform on which the animal's body could rest. Attached to the shaft of the gauge was a 1/16 inch diameter brass rod formed into the shape of a triangle. The brass rod provided the fixture which the mouse grasped.

Procedure. Each mouse was held by the base of the tail and lowered toward the brass triangle and allowed to grasp it with its forepaws. The mouse was then pulled steadily by the tail away from the rod until the mouse's grip was broken. The lateral force exerted on the gauge at the time the grip was broken was recorded from the dial of the gauge. Each mouse was given three such trials approximately 3 min apart. The greatest lateral force of the three trials was used as the grip strength score for that mouse. There were ten mice in each dose group. Drugs (except succinylcholine and pancuronium, which were tested 10 min after administration) were administered SC 30 min prior to testing. Ethanol and dantrolene sodium were administered IG.

After drug administration, but prior to removal from the cage for testing, the mice were observed for signs of behavioral impairment. Apparent (gross) changes in the overall level of locomotion or other unusual effects were noted. Mice that exhibited an abnormal, uncoordinated gait were considered ataxic.

Drugs. The following drugs were evaluated: diazepam and midazolam (Hoffmann-LaRoche), baclofen (Ciba-Geigy), methocarbamol (Robins), dantrolene sodium (Norwich Eaton), pentobarbital sodium (Nembutal, Abbott), phencyclidine (National Inst. Drug Abuse), ketamine (Ketaset, Bristol), chlorpromazine (Smith Kline and French), (±)2-amino-7-phosphonoheptanoic acid (AP7, synthesized at Searle), clonidine (Boehringer), lithium chloride and ethanol (Aldrich), caffeine (Eastman Kodak), morphine sulfate (Mallinkrodt), *d*-amphetamine sulfate, succinylcholine and pancuronium bromide (Sigma).

Data analysis. Statistical trend tests were used to assess the dose-related effects of each drug. Comparisons of drug doses with vehicle were determined sequentially, starting with the highest dose and eliminating each dose in sequence until a significant ($P < 0.05$) difference from vehicle was not obtained; lower doses were not analyzed. This is a powerful method for the determination of the lowest effective dose for each drug (Tukey et al. 1985). The *P*-value for the highest dose was two-tailed, and *P*-values for subsequent doses were one-tailed in the direction determined by the highest dose.

Results

Vehicle-treated control mice were tested concurrently with each of the drugs evaluated. These animals readily grasped and held the rod of the test apparatus. The mean forelimb grip strength values for 30 (test drug vehicle plus pilot test) groups of 10 vehicle-treated mice ranged from 99 to 149 g with a mean of 120 (± 2.7) g: the distribution of these means is illustrated in Fig. 1. Variability between groups of vehicle-treated mice within test days was low, while variability across test days was greater. The latter variability, at least as pertains to the extremes in Fig. 1, is accounted for largely by differences in the body weights of the mice. Lighter groups of mice had lower, and heavier groups of mice had higher forelimb grip strength values. Therefore, body weights were monitored to maintain consistency across test groups within test days.

Peripherally and centrally acting drugs of various types were evaluated for effects on mouse forelimb grip strength. The results, summarized in Table 1, are expressed as percentages of the concurrently tested vehicle control grip strength means (also listed in the table). Doses that differed significantly from vehicle are indicated.

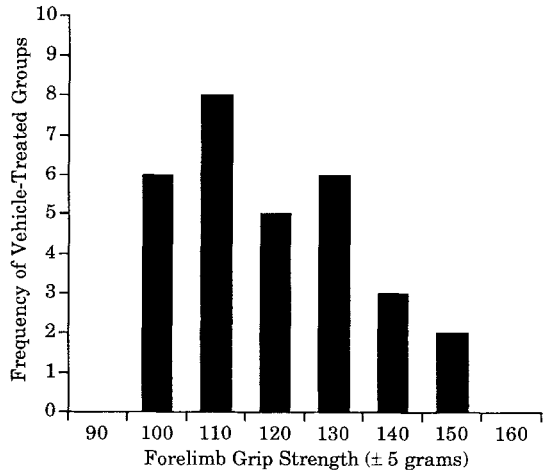

Fig. 1. Frequency distribution of forelimb grip strength means for groups of 10 mice treated with various drug vehicles

Immediately prior to forelimb grip strength testing, all mice were observed for signs of behavioral impairment. In general, mice treated with the muscle relaxants, diazepam, midazolam, baclofen, methocarbamol and dantrolene, tended to exhibit mild ataxia and reduced locomotion at doses which significantly reduced forelimb grip strength. The exception was AP7-treated mice, which did not exhibit signs of behavioral impairment. The neuromuscular blocking agents, pancuronium and succinylcholine, also reduced forelimb grip strength at doses which did not affect behavior or respiration. Ethanol, pentobarbital and chlorpromazine produced signs of marked sedation (i.e., marked ataxia, ptosis and severely depressed locomotion) at the doses which were found to reduce grip strength. Phencyclidine and ketamine also produced gross behavioral impairments at doses which reduced grip strength. However, the impairments differed in that these drugs produced hyperactivity and tremors along with severe ataxia and impairments in coordinated movements.

The stimulants, amphetamine and caffeine, produced hyperactivity at doses which were found to increase forelimb grip strength. Morphine also produced hyperactivity and Straub tail over the dose range tested, but failed to affect forelimb grip strength. Lithium chloride did not produce signs of behavioral impairment or alter forelimb grip strength at the doses tested. Mice treated with clonidine exhibited ataxia, piloerection and reduced locomotion at the higher doses, but no effect was seen on grip strength.

Discussion

In the present study, a variety of centrally and peripherally acting drugs were examined to determine whether effective muscle relaxants differentially reduce

Table 1. The effects of various centrally and peripherally acting drugs on forelimb grip strength in mice. Results are expressed as percentages of the grip strength values of concurrently tested vehicle-treated mice. *$P < 0.05$ vs vehicle; **$P < 0.01$ vs vehicle

Drug	Dose (mg/kg)	Route	% of vehicle (SE) Forelimb grip strength
Vehicle			(99 g)
Amphetamine	1.0	SC	117.2 (7.1)
	3.2		136.2 (7.2) **
	5.6		135.8 (8.0) **
Vehicle			(108 g)
(+/−)-AP7	17.8	SC	87.9 (5.8)
	32		85.1 (5.4) *
	56		80.4 (5.2) **
	100		67.0 (4.7) **
Vehicle			(109 g)
Baclofen	5.6	SC	105.0 (4.1)
	7.8		110.5 (6.0)
	10		61.8 (5.9) **
	17.8		42.3 (10.5) **
Vehicle			(101 g)
Caffeine	50	SC	111.9 (4.9)
	100		141.3 (7.1) **
	150		134.8 (7.3) **
	200		118.4 (5.2) **
Vehicle			(131 g)
Chlorpromazine	1.0	SC	86.2 (5.2) *
	1.78		72.4 (5.2) **
	3.2		56.3 (5.0) **
Vehicle			(117 g)
Clonidine	0.001	SC	91.9 (3.9)
	0.01		100.4 (5.2)
	0.1		108.5 (5.7)
	0.3		93.2 (3.4)
Vehicle			(149 g)
Dantrolene	5.6	IG	90.4 (3.7)
	10		74.5 (5.2) **
	17.8		70.8 (4.1) **
	32		61.1 (4.5) **
Vehicle			(101 g)
Diazepam	0.32	SC	94.1 (6.3)
	0.56		92.1 (3.1)
	1.0		74.7 (5.1) **
	1.78		65.3 (5.3) **
	3.2		59.4 (5.1) **
	5.6		40.1 (6.0) **
Vehicle			(140 g)
Ethanol	560	IG	95.1 (4.2)
	1000		90.3 (3.9)
	1780		92.1 (5.6)
	3200		43.7 (12.3) **
Vehicle			(133 g)
Ketamine	3.2	SC	100 (5.2)
	5.6		89.4 (2.5)
	10		83.4 (5.1) **
	17.8		73.2 (4.1) **
Vehicle			(117 g)
Lithium chloride	32	SC	90.2 (4.7)
	56		104.1 (4.4)
Vehicle			(131 g)
Methocarbamol	300	SC	98.9 (5.1)
	400		86.6 (6.3)
	500		64.1 (8.4) **
Vehicle			(146 g)
Midazolam	0.56	SC	83.5 (3.4) *
	1.0		80.4 (6.1) **
	1.78		67.0 (5.7) **
Vehicle			(131 g)
Morphine SO_4	1.0	SC	90.0 (5.3)
	3.2		106.9 (4.2)
	5.6		108.1 (3.4)

Table 1. (continued)

Drug	Dose (mg/kg)	Route	% of vehicle (SE) Forelimb grip strength
	10		99.2 (4.7)
	17.8		105.4 (3.5)
	32		111.8 (4.3)
	56		99.3 (4.9)
Vehicle			(125 g)
Pancuronium	0.03	SC	99.2 (3.3)
	0.06		88.3 (8.8)
	0.1		75.9 (3.1) **
Vehicle			(114 g)
Pentobarbital Na	10	SC	111.9 (2.9)
	17.8		94.3 (8.2)
	25		95.6 (6.9)
	32		56.4 (8.7) **
Vehicle			(110 g)
Phencyclidine	1.0	SC	106.4 (4.5)
	3.2		79.5 (10.8) *
Vehicle			(103 g)
Succinylcholine	0.6	SC	104.9 (4.7)
	1.0		69.3 (9.1) **
	1.3		30.1 (7.8) **

forelimb grip strength. The results extend the findings of previous reports (Van Reizen and Boersma 1969; Meyer et al. 1979), which showed that mouse forelimb grip strength is susceptible to reduction by some muscle relaxants, and suggest that the grip strength test could be a useful screening test for novel muscle relaxant drugs. In addition, the present data show that forelimb grip strength is susceptible to pharmacologic manipulation in both directions (i.e., it can be either decreased or increased).

All of the centrally acting muscle relaxants tested, diazepam, midazolam, baclofen and methocarbamol produced dose-dependent decreases in grip strength. In addition, AP7, an antagonist at the N-methyl-D-aspartate receptor which has been shown to exhibit muscle relaxant effects in genetically spastic rats (Turski et al. 1985), also reduced forelimb grip strength in a dose-dependent manner. Similarly, the peripherally acting muscle relaxant, dantrolene, which acts directly on muscle tissues (Ellis et al. 1973), and the neuromuscular blocking agents, pancuronium bromide and succinylcholine, were found to reduce forelimb grip strength in a dose-dependent manner.

Several sedative-hypnotic and anesthetic drugs were evaluated. Ethanol, pentobarbital Na, phencyclidine, ketamine, and the major tranquilizer, chlorpromazine, were found to reduce forelimb grip strength. These effects were generally seen at doses which produced behavioral impairments.

The stimulants, amphetamine and caffeine, were found to increase forelimb grip strength in a dose-dependent manner. This enhancement is independent of any effect on locomotion because morphine, at doses (10–56 mg/kg) which stimulate locomotor activity in mice (Kuschinsky and Hornykiewicz 1974), did not affect forelimb grip strength, and because phencyclidine and ketamine, at doses that increased locomotion, reduced grip strength. Therefore, the enhancement of grip strength by amphetamine and caffeine appears to be a specific effect on muscle tone.

In order to investigate the possibility that a behavioral measure, such as forelimb grip strength, might be affected by non-specific effects, lithium chloride and clonidine were evaluated. Lithium chloride, which produces intestinal malaise and is an effective toxic agent for the formation of learned taste aversions (Nachman and Ashe 1973), did not affect forelimb grip strength. In addition, clonidine, at doses which alter blood pressure (Ozawa et al. 1977), reduce body temperature (McLennan 1981) and reduce locomotion (Delini-Stula et al. 1979), failed to alter grip strength. Therefore, while the grip strength test is sensitive to the effects of skeletal muscle relaxants, it is insensitive to the effects of drugs which do not affect muscle tone.

Assessment of forelimb grip strength has several advantages over other simple behavioral tests which are currently used to infer muscle relaxation. It is a rapid, easily conducted test which does not require that the animals be trained on, or familiarized with, the apparatus. Mice held by the tail readily grasp the brass rod. In contrast to the all-or-none test procedures such as the rotating rod (Dunham and Miya 1957) or the inverted screen (Coughenour et al. 1977) the results of the current procedure provide quantitative estimates of the animals' performance; also in contrast to the former procedures, grip strength can be either enhanced or impaired. Another advantage is that, unlike the blockade of mouse Straub tail test (Ellis and Carpenter 1974), grip strength evaluation does not require the presence of another drug.

In conclusion, the current procedure provides a specific method for the quantitative assessment of forelimb grip strength in the mouse. This method appears to be capable of discriminating muscle relaxant from other pharmacological effects, and therefore, appears to offer a useful screening procedure for the identification of potential muscle relaxant activity of novel compounds. That is, by use of this method, compounds likely to exhibit muscle relaxant activity could be rapidly identified for verification by subsequent testing. Like all screening procedures, the forelimb grip strength test is liable to identify false positives. Sedatives with relatively little muscle relaxant effects, drugs that produce peripheral neuropathies and drugs that may affect the animals' motivation to grip the bar would be potential false positives. Subsequent testing,

in assays that specifically assess muscle function or activity at the neuromuscular junction, would be used to differentiate active compounds from false positives.

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