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SOME PHARMACOLOGICAL STUDIES ON THE SPASTIC MOUSE

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1 Full-wave rectification and integration of the EMG signal recorded from the hamstring muscles of the spastic mouse was used to evaluate the actions of a variety of drugs on the muscle rigidity of these mutants, animals in which no histological lesion has yet been found.

2 Profound and long-lasting muscle relaxant responses were consistently observed upon the injection of diazepam (2 mg/kg, i.p.) and flunitrazepam (2 mg/kg, i.p.). Such responses were always greater than those obtained upon injection of 40% (v/v) propylene glycol (10 ml/kg) alone, the vehicle for the benzodiazepines.

3 The muscle relaxant action of a low dose (0.25 mg/kg, i.p.) of the benzodiazepine Roll-6896 was not shared by the same dose of its enantiomer Roll-6893.

4 Profound and long-lasting muscle relaxation was caused by sodium valproate (696 mg/kg, i.p.). Consistent muscle relaxant responses were also observed upon the injection of pentobarbitone (30 mg/kg, i.p.), but not phenobarbitone (30 mg/kg, i.p.).

5 Other drugs that had little or no detectable effect on the muscle rigidity of the spastic mouse included diphenylhydantoin (30 mg/kg, i.p.) and bromocriptine (10 mg/kg, s.c.) while, in some animals, benztropine (2 mg/kg, i.p.) and baclofen (10 mg/kg, i.p.) increased muscle rigidity.

6 The development of full muscle relaxant responses to flunitrazepam (2 mg/kg, i.p.) and to sodium valproate (696 mg/kg, i.p.) was shown to depend upon mild warming of the animals with radiant heat, a procedure which can increase muscle spindle afferent input to the spinal cord.

7 The results suggest a hyperactivity of stretch reflexes in the spastic mouse, ameliorated selectively by those drugs that enhance the GABA-mediated presynaptic inhibition of such pathways.

Introduction

Mice homozygous for the spastic (*spa*) gene display skeletal muscle rigidity and tremor (Chai, 1961), especially when given a vestibular stimulus and deprived of tactile input from the limbs. Examination of the central nervous system (CNS) of these mutants by conventional light microscope techniques has, however, failed to reveal a lesion (Chai, Roberts & Sidman, 1962), a failure that we have confirmed in an extensive series of Golgi studies. Accordingly, we have considered the possibility that the *spa* gene is responsible for some biochemical defect in neurotransmitter function which is not associated with a gross structural abnormality.

Administration of amino-oxyacetic acid, a γ-aminobutyrate transaminase (aminobutyrate transaminase; EC 2.6.1.19) inhibitor which increases the concentration of γ-aminobutyric acid (GABA) in the CNS, was found to ameliorate some of the symptoms of the spastic mouse: the duration of the spasms induced by laying the animals on their backs was reduced as was the intensity of the tremor felt by holding them up by the tail, while their placing responses and ability to walk over a smooth surface were improved (Chai *et al.*, 1962). Concentrations of GABA in the brains of spastic mice have, however, been reported to be within the normal range (Chai *et al.*, 1962) and there appear to be no significant differences in the activities of glutamate decarboxylase (EC 4.1.1.15) and γ-aminobutyrate transminase (Chatterjee & Hechtmann, 1977), enzymes involved in the synthesis and catabolism of GABA, respectively.

In a further attempt to elucidate the nature of the lesion in the spastic mouse, we have treated these animals with several drugs that are thought to mimic or potentiate the actions of GABA in the CNS (Johnston, 1978), in order to see whether or not such agents are able to reduce the muscle rigidity. A number of other agents used clinically in the treatment of muscle rigidity and tremor have been included for comparison. Some of the results presented here have been communicated to the Physiological Society (Biscoe & Fry, 1980).

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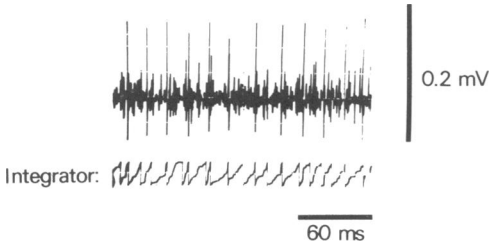

Figure 1 Electromyogram (EMG) recorded from the hamstring muscles of a spastic mouse, as displayed on an oscilloscope; lower trace shows the full-wave rectified and integrated signal used to quantitate EMG activity.

Methods

Under light ether anaesthesia, mice were placed in a harness constructed from two (approx. 18 mm long) split pieces of (15 mm i.d., 3 mm thick) rubber tubing. Holes had been punched in this harness through which the limbs could protrude and each piece of tubing was tied by a loop of thread behind the back of the animal, so that it was gently but firmly restrained and could be suspended in a horizontal position with the limbs freely exposed. One hindlimb was shaved and two uninsulated, electrolytically-sharpened tungsten needles (0.1 mm diameter; 10 mm long) pushed into the hamstring muscles, about 3 mm apart. A third needle inserted under the skin above the muscles acted as a ground electrode. The wires connecting these needles to the preamplifier were kept slack and their insulating collars glued together with a drop of cyanoacrylic adhesive, at the point of insertion into the limb, to prevent them being dislodged by any movement of the animal.

During the initial ether anaesthesia, all mice were fitted with intraperitoneal or subcutaneous cannulae of Portex nylon (00 gauge) tubing, the latter having a tip made from a 25G syringe needle that could be inserted under the skin at the back of the neck. Core temperature was monitored by a fine thermocouple inserted 20 mm into the rectum and in some experiments another thermocouple was placed subcutaneously above the hamstring muscles (where it replaced the tungsten ground electrode). Unless otherwise stated, the mice were warmed continuously by an (275W) infra-red heating lamp, positioned 550 mm above the harness. They were given access to water, were able to defaecate and urinate and showed no signs of stress during the procedure.

The EMG was recorded differentially through a Medelec Preamplifier PA 467/15 and Biological Amplifier AA6 Mk.II for continuous display on an oscilloscope (Figure 1) and a Minograph chart recorder (Figure 2). Full-wave rectification and integration of this EMG signal by a Medelec Integrator 16 gave a series of pulses at a frequency proportional to

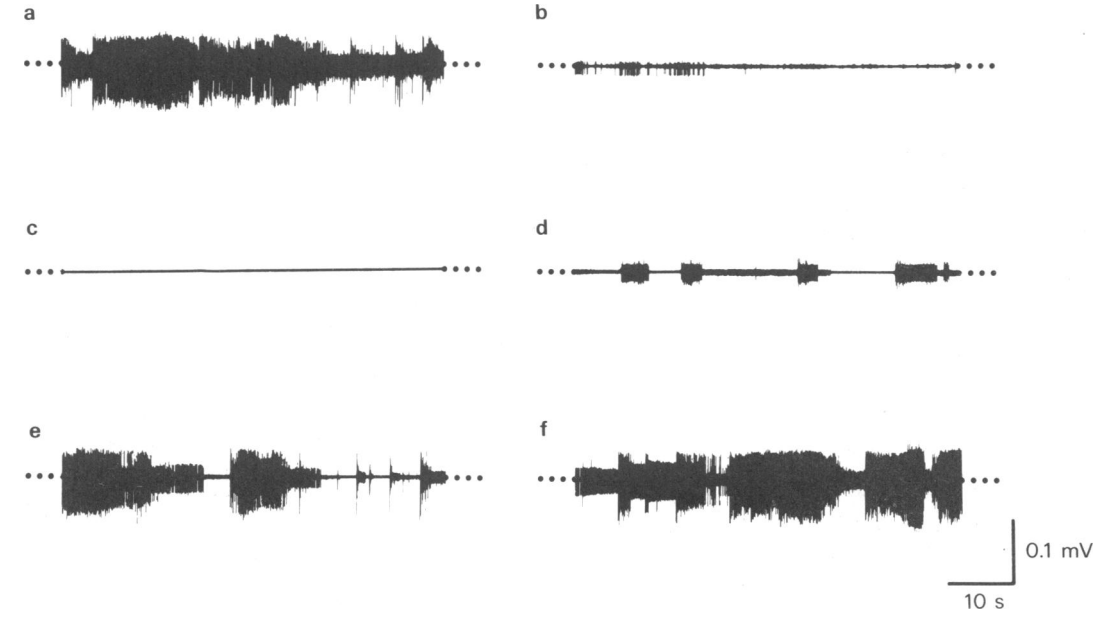

Figure 2 Mingograph chart recordings of EMG activity in a spastic mouse (a) 30 min before; (b) 20 min, (c) 30 min, (d) 3.5 h, (e) 6.0 h and (f) 6.5 h after injection of sodium valproate (696 mg/kg, i.p.). The full-wave rectified and integrated EMG activity recorded throughout this experiment and summed over successive 5 min intervals is illustrated in Figure 5(b).

the electrical activity of the muscle (see Figure 1). These pulses were counted automatically and used to provide a voltage/time integral which, for presentation of results, was summed over sucessive 5 min periods (Figure 3). Readings taken during the first 1–2 h after the initial ether anaesthesia, when EMG activity tended to decline, were discarded and the subsequent 2 h used as a control period before treatment of the animal. Changes, if any, in the EMG could then be expressed as a percentage of the mean activity recorded in this control period. The significance of changes in EMG activity was evaluated by using the Mann-Whitney U-test (Siegel, 1956). For most experiments the 60 min period following injection of the drug vehicle alone was compared with both the preceding 60 min control period and the subsequent 60 min period that followed injection of the drug itself. Departures from this practice are indicated in Results.

A total of 39 spastic mice, of either sex, weighing 19–30 g and 9–52 weeks old were used for this study. Owing to limitations on the size of the colony, some mice had to be used for experiments more than once, but were left for intervals of at least three weeks between treatments. Each drug, moreover, was tested at least twice in a naïve mouse and no gross differences observed between the responses of these animals and those that had received drugs on previous occasions. As a further precaution against drug interactions, mice were not injected more than once with the barbiturates or diphenylhydantoin, drugs for which metabolizing enzymes might be induced.

The following drugs were employed: the 1,4-benzodiazepines, diazepam, flunitrazepam, Roll-6893 and Roll-6896, the latter two compounds being the (−)- and (+)-isomers of 7-nitro-5-(-2-fluorophenyl)-1,3-dihydro-1,3-dimethyl-2H-1,4-benzodiazepin-2-one, respectively (all from Roche Products) in an aqueous solution of 40% (v/v) propylene glycol (BDH, laboratory reagent grade); bromocriptine (2-bromo-α-ergocryptine, CB154; Sandoz Products) dissolved at 100 mg/ml in an aqueous solution of 70% (v/v) ethanol containing an equal amount of (+)-tartaric acid before dilution to a final concentration of 5 mg/ml with distilled water; baclofen (CIBA laboratories), benzotropine mesylate (Merck, Sharp & Dohme), sodium valproate (sodium di-*n*-propylacetate; Reckitt & Colman Pharmaceutical Division), and pentobarbitone sodium (Abbott Laboratories) all in 150 mM NaCl solution; diphenylhydantoin sodium (phenytoin sodium; Sigma) in 150 mM NaCl solution (pH, 8.0); phenobarbitone (Sigma) in 150 mM NaCl solution (pH, 11.0). Intraperitoneal and subcutaneous injections were given in volumes of 10 ml/kg and 2 ml/kg, respectively, and the cannulae flushed with 0.1 ml of 150 mM NaCl solution after each injection. Dosages are expressed as the amount of free acid or base administered, for all drugs except sodium valproate.

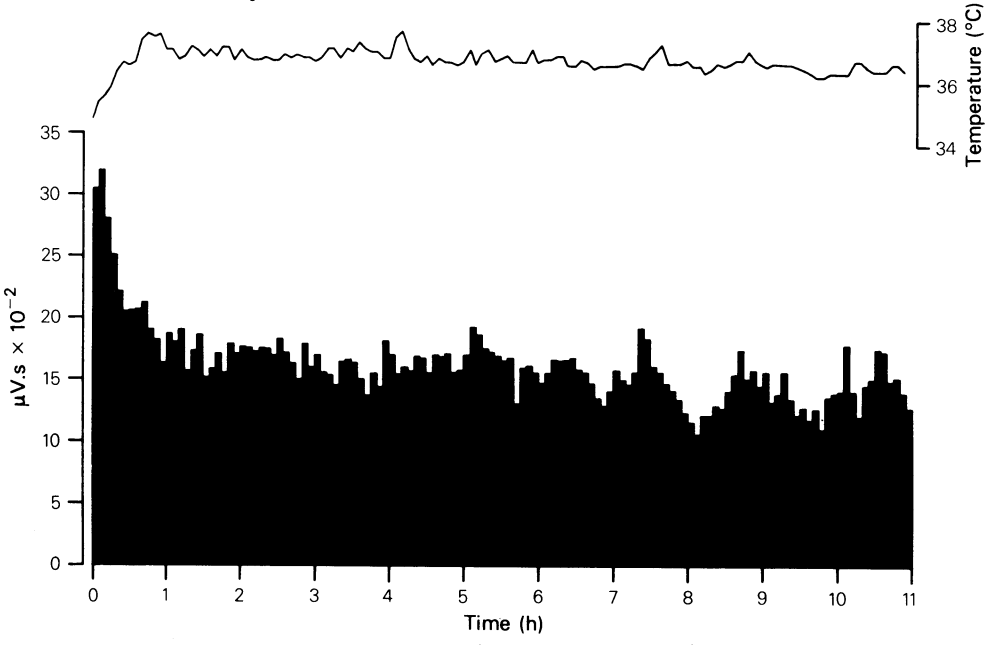

Figure 3 Full-wave rectified and integrated EMG activity as summed over successive 5 min intervals in a spastic mouse. Recording began 16 min after the initial ether anaesthesia necessary to install the animal in a rubber harness and insert the tungsten needle electrodes. Upper trace shows rectal temperature readings.

Results

In normal mice, continuous and stable recordings of EMG activity cannot be made over long periods of time because of intermittent and irregular changes in muscle tone. By way of contrast, when recordings are attempted from spastic mice in the manner described here, intense EMG activity persists in the hamstring muscles for at least 12 h. A whole body tremor also persists in these animals, at a frequency of approximately 30 Hz, but was not evaluated in the present experiments.

Continuous warming of these mice with an infra-red lamp kept core temperatures within the normal range (see Figure 3), but was not sufficient to prevent a certain degree of hypothermia upon injection of the following drug dosages (ranges from maximal rectal temperature decrease shown, $n > 4$): propylene glycol (40% v/v, 10 ml/kg, i.p.) 0.3–1.1°C; diazepam (2 mg/kg. i.p.) 0.7–2.9°C; flunitrazepam (2 mg/kg, i.p.) 1.0–3.5°C; Roll-6893 (0.25 mg/kg, i.p.) 0.2–1.7°C; Roll-6896 (0.25 mg/kg, i.p.) 1.1–2.6°C; pentobarbitone (30 mg/kg, i.p.) 0.2–1.0°C; sodium valproate (696 mg/kg, i.p.) 0.6–1.7°C; diphenylhydantoin (30 mg/kg, i.p.) 0.7–1.3°C; bromocriptine (10 mg/kg, s.c.) 0.8–3.0°C; baclofen (10 mg/kg, i.p.) 1.0–1.9°C.

Typical effects of different drugs on the EMG activity of the hamstring muscles in spastic mice that were warmed continuously with an infra-red lamp are shown in Figures 4, 5 and 6. The 1, 4-benzodiazepines (diazepam, flunitrazepam, Roll-6893 and Roll-6896) were dissolved for injection in an aqueous solution of propylene glycol (40%, v/v).

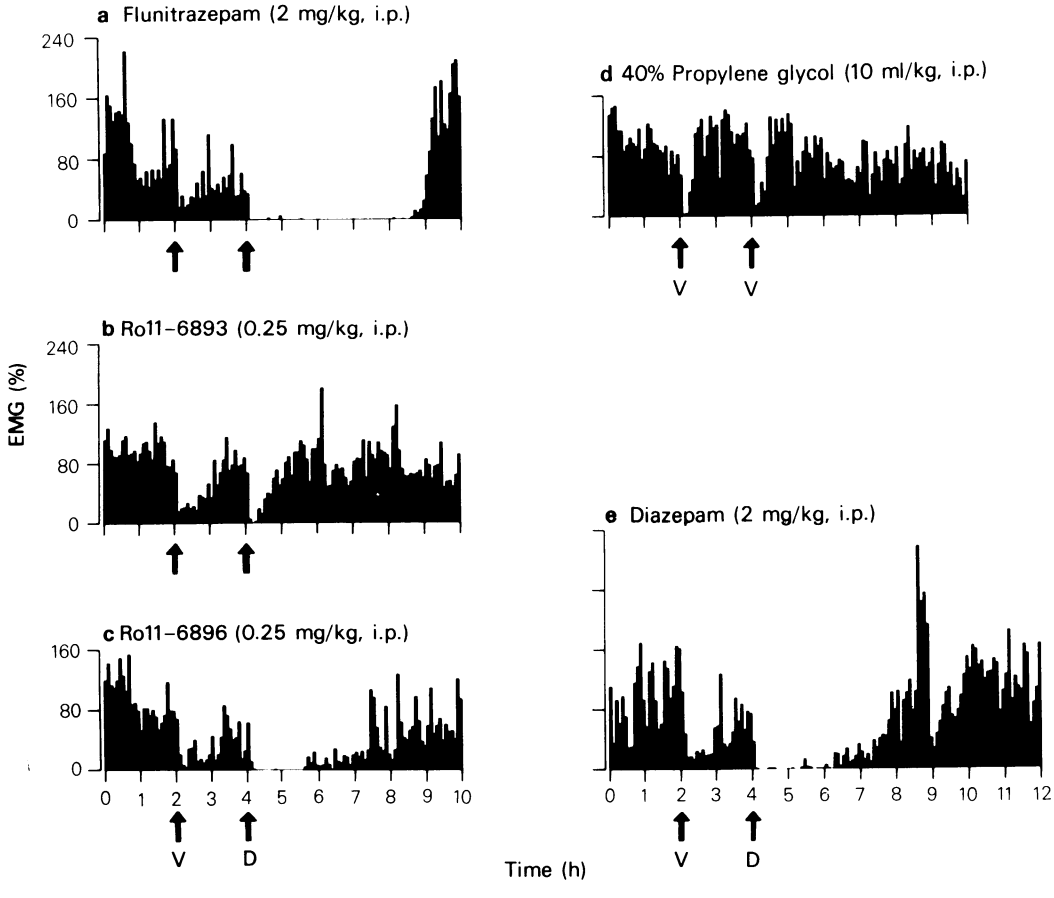

Figure 4 Muscle relaxant action of various benzodiazepines in the spastic mouse, as shown by full-wave rectified and integrated EMG activity, summed over 5 min intervals. The EMG activity is expressed as a percentage of that occurring during the 2 h control period before injection of the drug vehicle (V). (d) Two injections of the drug vehicle, 40% (v/v) propylene glycol (10 ml/kg, i.p.) alone. Benzodiazepines were injected (D) 2 h after an injection of the vehicle alone: (a) flunitrazepam (2 mg/kg, i.p.); (b) Roll-6893 (0.25 mg/kg, i.p.); (c) Roll-6896 (0.25 mg/kg, i.p.); (e) diazepam (2 mg/kg, i.p.).

This solvent alone (at 10 ml/kg, i.p.) caused a significant ($P<0.001$) muscle relaxation in 22 of the 27 mice tested (see Figure 4). The muscle relaxant effect of propylene glycol became apparent within 10 min of injection, but only persisted for about 20–30 min and did not increase upon a subsequent injection 2 h later ($P>0.05$; 4 mice), indicating a lack of cumulative effects of this drug vehicle in the present experiments. Muscle relaxant effects of propylene glycol could, moreover, be clearly distinguished from the profound and long-lasting relaxation induced by injection of diazepam (2 mg/kg, i.p.; $P<0.001$; 4 mice) or flunitrazepam (2 mg/kg, i.p.; $P<0.001$; 4 mice). When given at these dosages, both of the benzodiazepines depressed EMG activity below 1% of that recorded in the control period before injection of the drug vehicle, a relaxant effect that occurred within 10 min of injection and persisted for 70–290 min (Figure 4).

Injection of a low dose (0.25 mg/kg, i.p.) of Roll-6893 had a slight muscle relaxant effect which, in 4 of the 5 animals tested, was not significantly different ($P>0.05$) from that seen after the drug vehicle alone. The same dose of its (+)-enantiomer Roll-6896, in contrast, induced a clear relaxation ($P<0.001$; 4 mice) within 10 min of injection, that persisted for 55–95 min (Figure 3). At a higher dosage (1 mg/kg; 2 mice) Roll-6893 was found to cause a relaxation for 35–50 min, greater ($P<0.01$) than that seen after injection of the drug vehicle alone, but always of shorter duration than that (170–235 min) elicited by the same dose of Roll-6896 (results not shown).

Muscle relaxation was also observed within 10 min of the injection of pentobarbitone (30 mg/kg, i.p.; 5 mice; $P<0.005$ for comparison of 40 min post-injection periods). This response persisted for 20–35 min, at which time EMG activity was depressed to less than 23% of that recorded in the control period before injection of the saline vehicle alone; recovery was rapid (see Figure 5). A similar dose of phenobarbitone (30 mg/kg i.p.) lacked any detectable muscle relaxant action ($P>0.05$) in 3 mice (see, for example, Figure 5) although in one other animal some slight relaxation ($P<0.05$) did appear to occur 40–110 min after injection.

Of the other drugs tested, the only one that had any consistent muscle relaxant action on the spastic mouse was sodium valproate (696 mg/kg, i.p.). All 4 mice tested with this drug became relaxed ($P<0.001$) within 10–20 min of injection and remained with EMG activity depressed below 12% of that recorded before injection of the saline vehicle alone for 135–200 min (Figure 5).

Injection of diphenylhydantoin (30 mg/kg, i.p.) induced a slight relaxation ($P<0.025$) in 2 out of the 4 mice tested but, although recordings were continued for 6 h, EMG activity never fell in any 5 min period below 24% of the mean of the 2 h control period before injection of the saline vehicle (Figure 5).

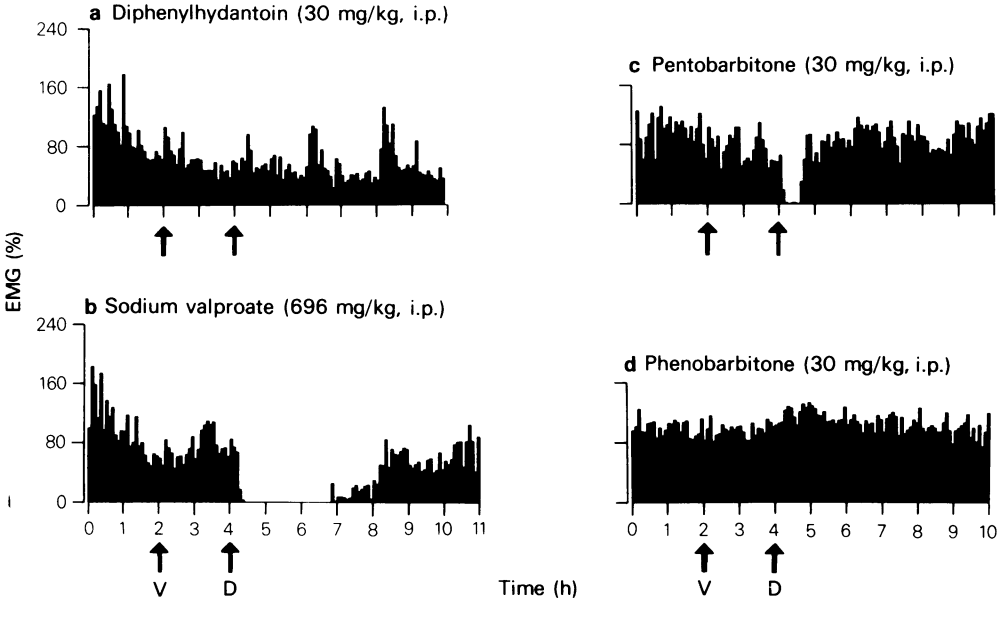

Figure 5 Comparison of the effects of (a) diphenylhydantoin (30 mg/kg, i.p.), (b) sodium valproate (696 mg/kg, i.p.), (c) pentobarbitone (30 mg/kg, i.p.) and (d) phenobarbitone (30 mg/kg, i.p.) on muscle rigidity in the spastic mouse. All drugs injected (D) 2 h after an injection (V) of the 150 mM NaCl vehicle (10 ml/kg) alone.

Bromocriptine was injected in a solution containing tartaric acid and ethanol (see Methods), which alone (at 2 ml/kg, s.c.) induced a slight but significant ($P<0.001$) muscle relaxation in 2 out of the 4 mice tested. Administration of bromocriptine (10 mg/kg, s.c.) caused no additional relaxation and EMG activity never fell below 20% of that recorded before injection of the drug vehicle. Indeed, as the 6 h of recording following drug injection progressed there was a tendency (in 3 of the 4 mice tested) for EMG activity to increase (see Figure 6).

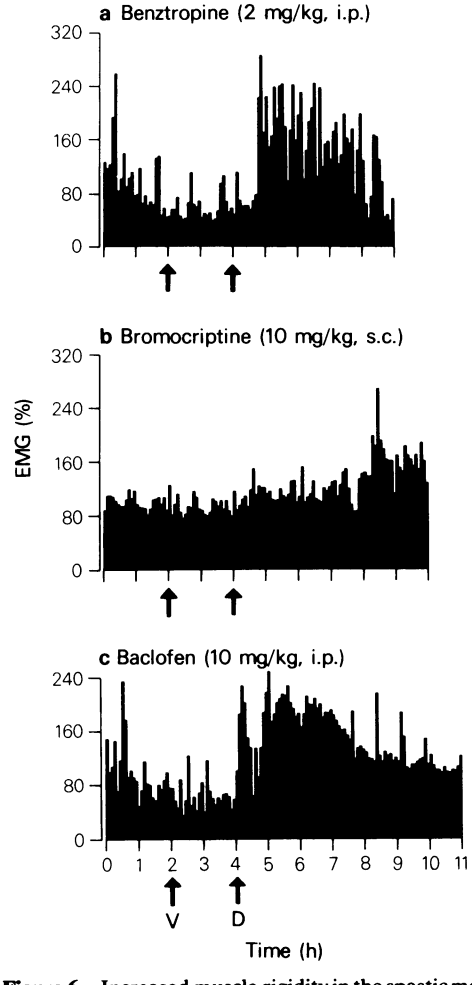

Figure 6 Increased muscle rigidity in the spastic mouse following injection of (a) benztropine (2 mg/kg, i.p.) and (c) baclofen (10 mg/kg, i.p.). Bromocriptine (b; 10 mg/kg, s.c.) also lacked any muscle relaxant action. All drugs injected (D) 2 h after an injection of the drug vehicle (V) alone, which was 150 mM NaCl (10 ml/kg) for benztropine and baclofen and a solution of tartaric acid in ethanol (2 ml/kg; see Methods) for bromocriptine.

Clearer increases in muscle rigidity ($P<0.001$ for comparison of 2 h following drug injection with 2 h following injection of the saline vehicle alone) were also observed in 3 of the 6 mice injected with benzotropine (2 mg/kg, i.p.). Such increases in EMG activity became apparent within 20–70 min and persisted for at least 2.5 h (see Figure 6). No muscle relaxant responses to this dosage of benzotropine were observed.

Another drug found to increase muscle rigidity was baclofen (10 mg/kg, i.p.); 3 out of 4 mice treated with this drug displayed significant ($P<0.001$) increases in EMG activity within 20 min of injection that did not abate until 45–325 min later (Figure 6).

Consistent muscle relaxant responses to the benzodiazepines and to sodium valproate were only observed in mice warmed continuously with an infrared lamp. This phenomenon was investigated in more detail by keeping mice at an ambient air temperature of 26–27°C and warming them at 90 min intervals with 30 min applications of the lamp, a procedure that caused reproducible elevations of both core temperature (range 1.1–2.2°C) and the subcutaneous temperature (range 2.0–4.6°C) above the hamstring muscles (see Figure 7). A total of 13 mice were warmed in this way, 9 of which displayed significant muscle relaxation, before treatment with any drugs or drug vehicles ($P<0.008$ for comparison of EMG activity when lamp on with activity during the preceding 30 min).

In the absence of radiant heat, the injection of 40% propylene glycol (10 ml/kg, i.p.) was found to induce a slight relaxation in 4 out of 8 mice tested ($P<0.01$ for comparison of 45 min post-injection period with preceding 45 min). When these 8 mice were injected with flunitrazepam (2 mg/kg, i.p.) 2 h after the injection of 40% propylene glycol alone, only 3 displayed a relaxation greater than that induced by this drug vehicle ($P<0.01$ for comparison of 45 min post-injection periods). However, upon the application of radiant heat to these 8 animals 45 min after drug injection, 5 showed muscle relaxant responses that were significantly greater than those observed upon warming 45 min after the injection of the drug vehicle alone ($P<0.008$ for comparison of 30 min warming periods). Clearly enhanced muscle relaxant responses to radiant heat were observed for at least 3 h after the injection of this dose of flunitrazepam (see Figure 7a).

Muscle relaxant responses to sodium valproate (696 mg/kg, i.p.) were also attenuated in the absence of radiant heat; only one of the 6 mice tested displayed a significant ($P<0.001$) reduction of EMG activity when the 45 min period after drug injection was compared with the 45 min following injection of the saline vehicle. Application of radiant heat to these 6 drug-treated annimals, however, evoked a

muscle relaxation significantly greater ($P<0.001$) in all cases than that seen 45–75 min after the injection of saline alone. As with flunitrazepam, the muscle relaxant action of radiant heat in these mice was enhanced by sodium valproate for at least 3 h after drug injection (see Figure 7b).

Discussion

Full-wave rectification and integration of the EMG recorded from the hamstring muscles of the spastic mouse provided a measure of the muscle rigidity in these animals against which a variety of agents could

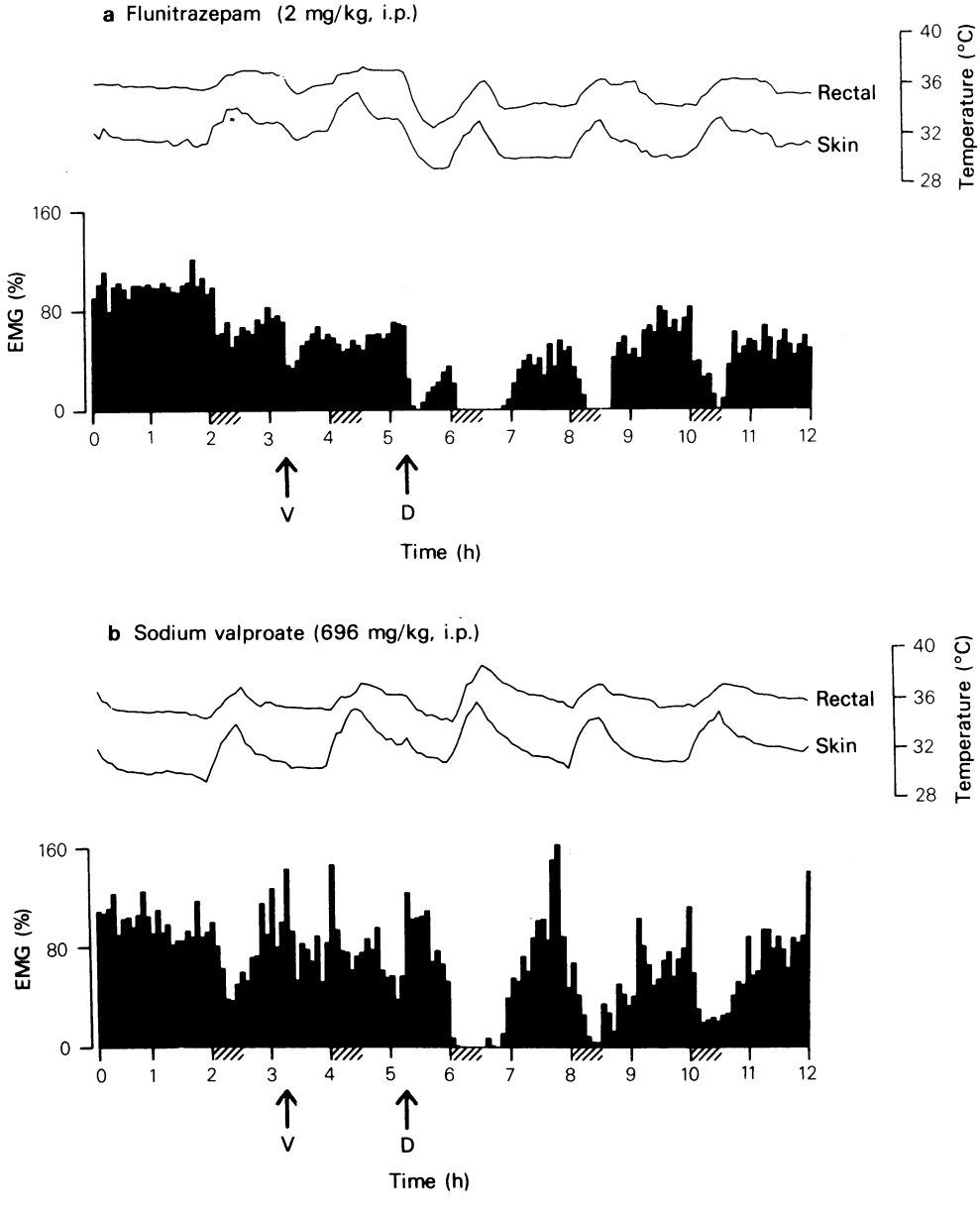

Figure 7 Effects of radiant heat (applied for the 0.5 h periods indicated by the striped bars under the abscissae) on rectal temperature, skin temperature and EMG activity in spastic mice treated with (a) flunitrazepam (2 mg/kg, i.p.) and (b) sodium valproate (696 mg/kg, i.p.). The EMG activity is expressed as a percentage of that occurring during the 2 h control period before the first application of radiant heat. Both drugs injected (D) 2 h after an injection (V) of their respective vehicles alone, as described in legends to Figures 4 and 5.

be tested. Of the drugs and drug vehicles used in this study, only propylene glycol, the benzodiazepines, sodium valproate and pentobarbitone provoked consistent and significant muscle relaxation in the spastic mouse.

Alleviation of the symptoms of the spastic mouse by amino-oxyacetic acid led Chai *et al.* (1962) to postulate that the spasticity in the mutants results from an imbalance of excitatory and inhibitory influences in some area of the CNS and that the elevated concentrations of GABA caused by treatment with this drug shift the balance in such a manner as to decrease the interference with normal function. Our present observation that benzodiazepines, sodium valproate and pentobarbitone ameliorate muscle rigidity in the spastic mouse is consistent with the above suggestion, since these drugs are known to mimic or potentiate the actions of GABA in the CNS (see below and Johnson, 1978).

The muscle relaxant action of diazepam has been attributed to its ability to enhance the prolonged, presynaptic inhibition of monosynaptic reflexes in the spinal cord, an effect seen in the pentobarbitone-anaesthetized or decerebrate, unanaesthetized cat after the systemic administration of this drug at dosages that have no detectable effect on the postsynaptic inhibition of motoneurones (Schmidt, Vogel & Zimmerman, 1967; Stratten & Barnes, 1971). Increases in such presynaptic inhibition can also be seen after injection of the aqueous solvent mixtures commonly used to dissolve diazepam for injection; 400% (w/v) glycofurol at 0.2–0.4 ml/kg, i.v. (Schmidt *et al.*, 1967) and 40% (v/v) propylene glycol at 2 ml/kg, i.v. (Stratten & Barnes, 1971). Earlier work has shown that pentobarbitone itself enhances the presynaptic inhibition of muscle afferents in the cat spinal cord (Eccles, Schmidt & Willis, 1963). The ability of propylene glycol, diazepam and pentobarbitone to cause muscle relaxation in the spastic mouse therefore invites consideration of the hypothesis that the stretch reflex loop is hyperactive in these animals owing, perhaps, to some defect in the presynaptic inhibitory control of muscle afferent terminals. Additional or alternative sites of action for these drugs in the spastic mouse cannot be excluded by the present experiments. Systemically administered pentobarbitone, for instance, has been shown to prolong hippocampal inhibitory postsynaptic potentials in the nitrous oxide/halothane-anaesthetized cat (Nicoll, Eccles, Oshima & Rubia, 1975) while diazepam appears to enhance the postsynaptic inhibition of neurones in the substantia nigra of the unanaesthetized cat (Haefely, Kulcsar, Möhler, Pieri, Polc & Schaffner, 1975) and to prolong the recurrent inhibition of pyramidal tract cells in the cerebral cortex of cats lightly anaesthetized with pentobarbitone or prepared *encéphale isolé* (Raabe & Gumnit, 1977). Both pentobarbitone and diazepam, moreover, have been reported to potentiate the basket cell inhibition of cerebellar Purkinje cells in pentobarbitone-anaesthetized and decerebrate cats (Curtis, Lodge, Johnston & Brand, 1976; Montarolo, Raschi & Strata, 1979) and to reduce the firing rate of these cells in intact, unanaesthetized preparations (Haefely *et al.*, 1975). The above observations have been contradicted, however, by Steiner & Felix (1976) who described an attenuation of the inhibitory effect of cerebellar Purkinje cells on neurones in the lateral vestibular nucleus of the nitrous oxide-anaesthetized cat following the intravenous injection of diazepam and by Julien (1972), who found such treatment to increase Purkinje cell firing rate in unanaesthetized animals.

Presynaptic inhibition of monosynaptic reflexes in the cat spinal cord is associated with a depolarization of the muscle afferent terminals (Eccles, Eccles & Magni, 1961), a phenomenon known as primary afferent depolarization (PAD) which is thought to be a response to GABA released from interneurones; GABA antagonists such as picrotoxin (Eccles *et al.*, 1963) and bicuculline (Curtis, Duggan, Felix & Johnston, 1971) decrease both presynaptic inhibition and PAD, while local microelectrophoretic application of GABA reduces the magnitude of the stimulating current necessary for evoking antidromic action potentials in single group Ia afferent terminals, in other words, increases their excitability (Sverdlov & Kozhechkin, 1975; Gmelin & Cerletti, 1976; Curtis, Lodge & Brand, 1977; Sastry, 1979). Concurrent with an increase and prolongation of presynaptic inhibition, diazepam is known to enhance PAD, as measured by an increase in the amplitude and duration of the dorsal root potential (Schmidt *et al.*, 1967; Stratten & Barnes, 1971; Polc, Möhler & Haefely, 1974) and the dorsal root reflex (Schlosser, 1971), together with an increased excitability of group Ia and group II muscle afferents (Stratten & Barnes, 1971).

The muscle relaxant action of diazepam and flunitrazepam in the spastic mouse was shared by a low dose of Roll-6896, but not by a comparable dose of its (−)-isomer Roll-6893, suggesting that these muscle relaxant responses are mediated by the newly discovered stereospecific binding sites for benzodiazepines in the CNS (Möhler & Okada, 1977; Squires & Braestrup, 1977). Non-specific actions of the benzodiazepines may also contribute to their muscle relaxant action, however, since increases in the dosage of Roll-6893 by less than one order of magnitude induced a significant relaxation in the spastic mouse, almost as great as that seen after injection of the lower dose of Roll-6896.

If benzodiazepines cause muscle relaxation in the spastic mouse by enhancing the action of GABA on

muscle afferent terminals, then these animals should respond to other drugs that are known to mimic or potentiate the actions of GABA in the CNS. This, as mentioned above, proved to be the case; the spastic mice displayed clear muscle relaxant responses to pentobarbitone and sodium valproate. The dosage of pentobarbitone (30 mg/kg, i.p.) was less than that normally employed for surgical anaesthesia in mice (see Green, 1979), including animals from our own C57BL6J spastic colony (Biscoe & Fry, unpublished). Pentobarbitone enhances the presynaptic inhibition of monosynaptic reflexes in the cat spinal cord (Eccles *et al.*, 1963; Miyahara, Esplin & Zablocka, 1966), and not only augments the actions of GABA on cultured mouse spinal cord neurones (Barker & Ransom, 1978), frog dorsal root ganglia (Nicoll, 1975) and group Ia afferent fibres in the cat ((Curtis & Lodge, 1978; Sastry, 1979), but also seems to possess GABA-mimetic properties in these preparations. In contrast to pentobarbitone, phenobarbitone injected at the same dosage failed to elicit clear muscle relaxant responses in the spastic mouse. Phenobarbitone is known to be less potent than pentobarbitone as an anaesthetic when administered intraperitoneally in the mouse (Gruber, Ellis & Freedman, 1944) and has also been found to be less potent than pentobarbitone at augmenting the actions of GABA on mouse spinal neurones in culture (MacDonald & Barker, 1978) and appears to have less effect on presynaptic inhibition in the cat spinal cord (Miyahara *et al.*, 1966). The clear muscle relaxant action of sodium valproate in the spastic mouse was seen after the injection of a dose that has been reported to increase the concentration of GABA in the brains of another strain of mouse (DBA/2) and to antagonize the audiogenic seizures of these animals, whilst causing no gross changes in motor behaviour (Anlezark, Horton, Meldrum & Sawaya, 1976). A muscle relaxant action of sodium valproate in the spastic mouse could be due to an elevated concentration of GABA in the CNS, as occurs after treatment of these mutants with amino-oxyacetic acid (Chai *et al.*, 1962), which is now known to increase both PAD and the presynaptic inhibition of monosynaptic reflexes in the cat spinal cord (Polc *et al.*, 1974). However, anticonvulsant effects of sodium valproate can be seen in the mouse after the injection of doses that have no apparent effect on the concentration of GABA in the CNS (Anlezark *et al.*, 1976). It is possible that other effects, such as the ability of this drug to augment responses to GABA in cultured mouse spinal cord neurones (MacDonald & Bergey, 1979) are important in the pharmacological actions of sodium valproate.

There was a lack of muscle relaxant responses to a variety of other drugs in the spastic mouse. Bromocriptine and benztropine, for example, two drugs that are used clinically for treating the rigidity and tremor of Parkinson's disease (Klawans, 1973; Calne, Teychenne, Claveria, Eastman, Greenacre & Petrie, 1974), failed to cause any clear relaxation in the present experiments. Bromocriptine is thought to stimulate cerebral dopamine receptors (Corrodi, Fuxe, Hökfelt, Lidbrink & Ungerstedt, 1973) and was administered to the spastic mutants at a dose reported to increase locomotor activity and stereotyped behaviour and to antagonize reserpine-induced catalepsy in the mouse (Johnson, Loew & Vigouret, 1976). The rigidity that results from treatment of rats with reserpine or physostigmine is associated with decreased γ- and increased α-mononeurone activity (Steg, 1964; Arvidsson, Roos & Steg, 1966) and has been attributed to a cholinergic/dopaminergic imbalance in the striatum (Arvidsson, Jurna & Steg, 1967). This rigidity is greatly diminished with doses of bromocriptine that have no detectable effect on the rigidity caused by intercollicular decerebration (Vigouret, Loew & Jaton, 1977). Bromocriptine therefore provides an interesting contrast to the benzodiazepines which, in the cat (Zbinden & Randall, 1974) are more effective against the rigidity attributed to γ-motoneurone hyperactivity following intercollicular decerebration than that caused by anaemic decerebration, in which α-motoneurone hyperactivity is said to predominate. Reserpine or physostigmine-induced rigidity in the rat can be reduced by atropine which, like bromocriptine, seems to restore the correct balance of α- and γ-motoneurone activity (Arvidsson *et al.*, 1966). In the spastic mouse, however, administration of the centrally-acting antimuscarinic agent benztropine, not only failed to reduce but sometimes exacerbated muscle rigidity, even when injected at a dosage known to have antimuscarinic actions in the mouse striatum (O'Keefe, Sharman & Vogt, 1970) and to antagonize oxotremorine-induced tremors in this species (Ahmed & Marshall, 1962). Central antimuscarinic actions of benztropine could, perhaps, increase muscle rigidity by depressing the synaptic excitation of the Renshaw cells involved in the recurrent inhibition of motoneurones (Curtis & Ryall, 1966). The lack of muscle relaxant responses to bromocriptine or benztropine does, nevertheless, suggest that the rigidity and tremor of the spastic mouse is not caused by a Parkinson-like lesion in the basal ganglia, a conclusion compatible with our previous failure to find a histological lesion in these areas.

Another drug which caused little or no muscle relaxation in the spastic mouse was diphenylhydantoin, injected at a dose reported to antagonize the seizures evoked by electroshock in mice (Knoefel & Lehmann, 1942). In contrast to the benzodiazepines (Haefely *et al.*, 1975) or the barbiturates (Swinyard,

Brown & Goodman, 1952) diphenylhydantoin appears to be inactive against bicuculline or metrazol-induced seizures in the mouse. In the cat, diphenylhydantoin has been reported to increase the supposedly GABA-mediated recurrent inhibition of pyramidal tract neurones in the cerebral cortex (Raabe & Ayala, 1976), but this effect was observed in pentobarbitone-anaesthetized animals and thus may not represent a primary action of the drug itself. Indeed, direct microelectrophoretic application of diphenylhydantoin to neurones in the dorsal raphe nucleus of chloral hydrate-anaesthetized rats has shown this drug to differ from the benzodiazepines in failing to enhance the depressant action of GABA (Gallager, Mallorga & Tallman, 1980). Systemic administration of diphenylhydantoin to decerebrate, unanaesthetized cats appears to have little effect on the spontaneous activity of cerebellar Purkinje cells (Haefely *et al.*, 1975) or on the inhibition of monosynaptic reflexes in the spinal cord, whether this inhibition is evoked by stimulating an antagonist muscle nerve or an adjacent dorsal root (Esplin, 1957). Synaptic depression following orthodromic volleys in such monosynaptic pathways is, however, deepened by diphenylhydantoin and there is an enhanced transmission failure during repetitive stimulation, together with a great reduction in post-tetanic potentiation. This latter action of diphenylhydantoin cannot be antagonized by metrazol or strychnine, agents used to block pre- and postsynaptic inhibition, respectively (see Schmidt, 1971) and is not shared by diazepam (Schlosser, 1971). The lack of a muscle relaxant response to diphenylhydantoin in the spastic mouse is consistent, therefore, with the hypothesis that only those drugs capable of enhancing GABA-mediated PAD decrease muscle rigidity in these animals.

Baclofen is a drug that was originally designed as a centrally-acting GABA receptor agonist and has been reported to be of use in the clinical treatment of spasticity (Birkmayer, Danielczyk & Weiler, 1967). Depressant effects of microelectrophoretically or systemically-applied baclofen on central neurones in the cat cannot, however, be readily antagonized by bicuculline (Curtis, Game, Johnston & McCulloch, 1974; Davies & Watkins, 1974; Fox, Krnjevic, Morris, Puil & Werman, 1978) and the drug does not compete for specific bicuculline-sensitive GABA binding sites or bicuculline binding sites on rat CNS membranes (see Johnston, 1978). When administered to the spastic mutants at a dosage known to antagonize the audiogenic seizures of DBA/2 mice (Meldrum, 1981) and to induce analgesia and decrease motor performance in normal mice (Cutting & Jordan, 1975), baclofen enhanced muscle rigidity. It is, therefore, of interest to note that baclofen has been found to reduce the PAD of both cuneate afferents (Polc & Haefely, 1976; Fox *et al.*, 1978) and group Ia terminals (Curtis & Lodge, 1978) in the cat. The ability of this drug to depress motoneuronal excitatory postsynaptic potentials without affecting resting membrane potential or conductance (Pierau & Zimmerman, 1973) now appears to be due to an inhibition of excitatory transmitter release, as seen in slices of guinea-pig cortex *in vitro* (Potashner, 1978), associated with a decrease rather than an increase in PAD (Fox *et al.*, 1978). These baclofen-induced reductions in transmitter output may involve a subclass of GABA receptors (Bowery, Hill, Hudson, Doble, Middlemiss, Shaw & Turnbull, 1980). A depression of transmitter release from the primary afferent terminals and/or interneurones involved in the generation of PAD (Curtis & Lodge, 1978) could explain the increased muscle rigidity observed in spastic mice following injection of baclofen. Such an explanation would only be tenable if EMG activity in the spastic mouse varied more according to the release of excitatory transmitter from the conditioning afferents and/or interneurones in the presynaptic inhibitory pathway than from the primary afferent terminals synapsing directly onto their homonymous motoneurones. This situation could, conceivably, arise if the muscle spindle afferents became hyperactive and would be exacerbated by any defects in the presynaptic inhibitory pathway.

Further indications of the importance of afferent input in the control of muscle tone in the spastic mouse were obtained by studying the effects of mild radiant heat, which was found to be essential for the expression of full muscle relaxant responses to the benzodiazepines and to sodium valproate. If the muscle relaxant responses of these drugs are due to a potentiation of presynaptic inhibition, then this synergistic effect of radiant heat is unlikely to be a direct result of increases in core temperature since, in the cat, the presynaptic inhibition of monosynaptic reflexes actually decreases upon warming of the spinal cord (Eccles *et al.*, 1961a; Eccles, Kozak & Magni, 1961b). Cutaneous warmth receptors in the hind limb are, moreover, unlikely to generate significant presynaptic inhibition of the myelinated afferents from muscle (see Schmidt, 1971). A more parsimonious explanation is that general warming of the hind limb musculature is also taking place, causing an increased discharge of the group I spindle endings (Mense, 1978) which would be expected to cause a powerful presynaptic inhibition of monosynaptic reflexes to the hamstring muscles and an increase in reciprocal and other postsynaptic inhibitions from antagonist and other muscles (see Schmidt, 1971). Diazepam is known to have no effect on the resting excitability of group Ia or group II muscle afferents (Stratten & Barnes, 1971) or of ulnar nerve terminals in the cuneate nucleus (Polc &

Haefely, 1976) and does not potentiate presynaptic inhibition or PAD at either of these locations if GABA has been depleted by thiosemicarbazide pretreatment (Polc *et al.*, 1974; Polc & Haefely, 1976). Adequate synaptic drive of the interneurones generating PAD would therefore be necessary for the full muscle relaxant action of diazepam and this was, perhaps, ensured in the present experiments by mild warming of the muscles. Similar results were obtained with sodium valproate suggesting that this drug might also potentiate synaptically-evoked PAD rather than alter the resting level of polarization of the muscle afferent terminals. Such a conclusion is consistent with observations of mouse spinal cord cells in culture, where sodium valproate is able to augment the actions of GABA at concentrations that have no detectable effect on resting membrane potential or conductance (MacDonald & Bergey, 1979).

Selective muscle relaxant responses of the spastic mice to a variety of drugs known to have as their common mode of action the ability to mimic or potentiate the actions of GABA in the CNS lead us to suggest that muscle rigidity in these animals is caused by a hyperactivity of the stretch reflex loop, ameliorated by drugs that enhance the GABA-mediated and prolonged, presynaptic inhibition of this monosynaptic pathway. While we cannot exclude possible supraspinal lesions, the present investigation does at least provide us with the first testable hypothesis for a disorder of function in the spastic mouse.

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