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Spasticity Phenomena Caused by N-Substituted Phenylalanine

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BLUM, B., R. SEGAL, G. SHTACHER AND B. SHOHAT. *Spasticity phenomena caused by N-substituted phenylalanine.* PHARMAC. BIOCHEM. BEHAV. 8(1) 19–23, 1978. – Symptoms which are pathognomonic of spasticity were induced in mice, rats, cats and squirrel monkeys by IV or IP injections of l– or d–N–carbobenzoxyphenylalanine (CBZPA) or N–t-butoxycarbonyl-l-phenylalanine in doses of about 100 mg/kg. Such effects are caused neither by the administration of similar substituents of other amino acids, nor by phenylalanine or the other amino acids themsleves. The spasticity-like symptoms characteristically appeared in the midst of elicited motor activity except in mice that showed them also at rest. The latency for the appearance of the symptoms was 4–7 min. These symptoms included asymmetric extensor spasms of the hindlimbs and flexor spasms in the forelimbs in mice, rats and cats; flexor spasms of the forelimbs and paresis of the hindlimbs in the squirrel monkeys. Hyperactive tendon tap reflexes were noted in all of these species as well as weak rightening reflexes, deficiency in antigravity reactions, and severe motor incoordination. Signs which are typical of spasticity observed in the monkey included the clasp knife sign, and in the cat, the lengthening reaction. In most cases, symptoms subsided within a week, but long-lasting incoordination was often observed. In few animals, severe massive rigidity evolved after a few days which sometimes terminated fatally. L–dopa showed no antagonistic effect against these CBZPA–induced spastic symptoms. In view of the specificity of the disturbances, a circumscribed brain pathology was assumed to be responsible. The possibility of interference with normal neurotransmitter function in specific brain regions is being considered.

Chemically induced spasticity Limb incoordination Movement disorders
1–N–carbobenzoxyphenylalanine Substituted amino acids

A NUMBER of amino acids and some of their derivatives have been shown to possess pharmacological activity at various regions of the central nervous system [17,24]. Some of these effects have been claimed to be manifestations of a neurotransmitter role played by the specific substance [2, 4, 9, 10, 15, 26]. In some cases a widespread nonspecific action was shown and a modulatory activity was assumed, including an action of coupling to transmission-related metabolism of neurons [11, 12, 37]. Effects of amino acid derivatives were assumed to be the result of interference with some neurotransmitter action [19].

While no evidence has been produced for phenylalanine as a putative neurotransmitter, it is of some significance that in phenylketonuria, a disease of disturbed phenylalanine metabolism, neurological dysfunction is evident [23]. Furthermore, in experimental hyperphenylalaninemia in which blood phenylalanine is abnormally elevated, evidence has been presented that phenylalanine interfered with its own and with other amino acid transport through the blood brain barrier [29,31], an effect which is deleterious to myelin formation [34].

A study was therefore undertaken on the neurological effects of N–substituted derivatives of phenylalanine, such as carbobenzoxy and other moieties. Alterations in overt behavior of a spastic nature produced by some of these derivatives in mice, rats, cats, and squirrel monkeys will be described.

METHOD

The study was carried out comparatively, using 6 squirrel monkeys, 30 cats, and over 400 rats (Sprague Dawley) and mice (Swiss) of both sexes. The animals received IP injections, except cats which also received IV injections of aqueous solutions of the sodium salts of the compounds (Table 1). Control groups of animals of each species received vehicle injections. Observations were made on the behavior of the animals during walking, running, and falling or jumping from a small height. The analysis of these effects was aided by movie films taken of the animals under these various conditions. Reflexes were tested prior to and after administration of the compounds.

In order to obtain some clues on possible mechanisms of action, the compounds listed in Table 1 were tried for agonistic or antagonistic effects. These included a number of amino acids including phenylalanine itself, carbobenzoxy

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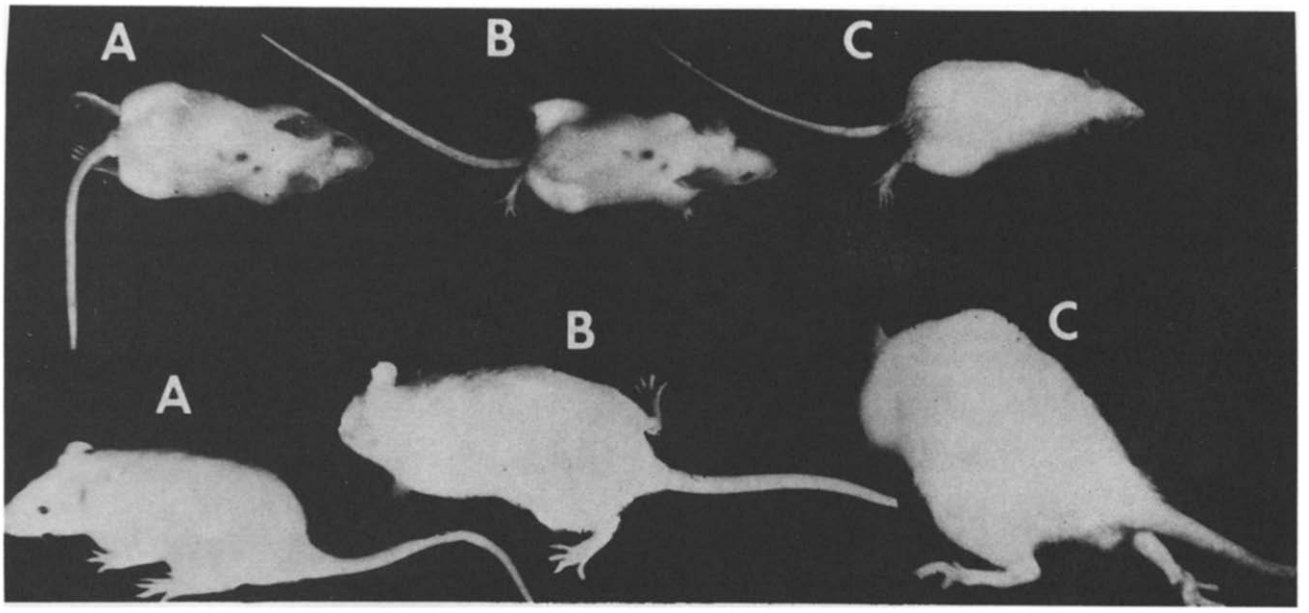

FIG. 1. Effects of IP injection of 100 mg/kg l-carbobenzoxy–N–phenylalanine (CBZPA) on mice and on rats. Upper row: Effects on mice. A. Extensor spasms of hindlimbs and flexor spasms of the forelimbs. In mice these appear also in rest. B. and C. The asymmetricity of these when they appear during elicited movement. Lower row: Effects on rats. A. The lack of overt symptoms at rest. B. and C. Asymmetric extensor spasms of the hindlimbs and flexor spasms of forelimbs appearing as the animal is stirred up to move.

FIG. 2. An illustration of the effects of CBZPA appearing during elicited movements in cats. (A) As the animal starts to run, its initial movements are normal; but then just as either of its hindlimbs reaches the degree of extension from which flexion should start, extensor spasms appear asymmetrically. (B) Flexor spasms in forelimbs: Animal is shown walking on dorsal side of paw (note right forelimb. Extensor asymmetric spasms of hindlimb are depicted (note left hindlimb).

TABLE 1

A LIST OF AMINO ACIDS, THEIR DERIVATIVES AND RELATED COMPOUNDS TESTED FOR AGONIST NEUROLOGICAL EFFECTS OR FOR AN ANTAGONISTIC EFFECT AGAINST THE CBZPA-INDUCED SPASTIC SYMPTOMS

1. Glycine	10. L-Tyrosine
2. N-Z-Glycine	11. N-Z-L-Tyrosine
3. L-Alanine	12. L-Dopa
4. N-Z-L-Alanine	13. L-Tryptophan
5. L-Phenyl Alanine	14. N-Z-L-Tryptophan
6. N-Z-L-Phenyl Alanine	15. L-Aspart c Acid
7. N-Z-D-Phenyl Alanine	16. N-Z-L-Aspartic Acid
8. N-t-BOC-L-Phenyl Alanine	17. L-Glutamic Acid
9. N-Ac-L-Phenyl Alanine	18. N-Z-L-Glutamic Acid

Z = Benzyloxycarbonyl (Carbobenzoxy); t-BOC = Tert. Butyloxycarbonyl (BOC derivative); Ac = Acetyl.

BOC, acetyl derivative of phenylalanine and of some of the other amino acids. In addition, l–DOPA was tried because of its well-known relation to dopamine and norepinephrine biosynthesis.

RESULTS

In mice, rats, cats and squirrel monkeys spastic symptoms were observed within 4–7 min following the administration of comparable doses of N–carbobenzoxy-l-phenylalanine (CBZPA) (50–100 mg/kg). These symptoms were evident only when motor activity was elicited, except mice which showed the symptoms also during rest.

Motor incoordination and spastic symptoms appeared as the animals tried to run: in about 40 mice, 40 rats, and 20 cats it was observed that just as the hindlimb completed its extension and was about to start flexion, it went into an extension spasm in a manner reminiscent of the lengthening reaction of spasticity (Figs. 1 and 2).

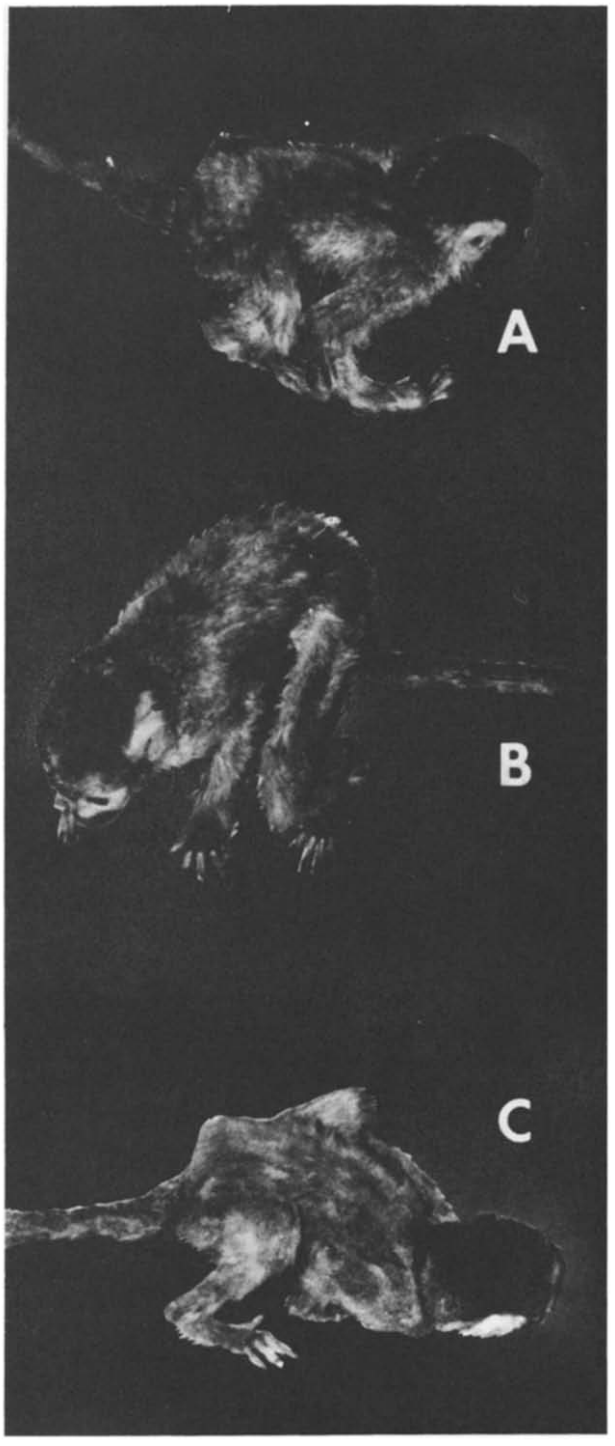

FIG. 3. Spasticity symptoms in forelimbs and paresis in hindlimbs in a squirrel monkey a few minutes after receiving an IP injection of CBZPA (100 mg/kg). A. The animal, stirred to move, assumed a gait typical of spasticity. B. and C. As the animal tried to run, flexor spasms appeared in the forelimbs (B) which made the monkey fall forward on its head (C).

In two squirrel monkeys, 70 mg/kg CBZPA produced only some paresis of the hindlimb. In contrast, 100 mg/kg CBZPA induced in six squirrel monkeys both the above paresis and also flexor spasticity of the forelimbs (Fig. 3). This spasticity was most clearly detected in the gait of the animal (Fig. 3A). Just as the monkies started to move, an excessive flexion of the forelimbs was observed, the animal assumed a typical spastic posture [27], and limb incoordination became prominent. As a result of the forelimb flexor spasm and hindlimb paresis, the animal's forward movement resulted in its falling on its head (Fig. 3B and C). In two monkeys the clasp knife sign, the primate spasticity equivalent of the cat's lengthening reaction was observed. A passive flexion of the elbow met with an increasing resistance until the limb reached a certain degree of flexion. Then resistance suddenly collapsed and the arm went into a full flexion.

In each of the above-mentioned species, CBZPA produced hyperactivity of stretch reflexes induced by tendon tapping. Rightening reactions were hampered by the appearance of spastic movements in their midst. Antigravity reactions were deficient, so that an animal dropped from a small height did not extend its limbs to break the fall, but landed on its abdomen (Fig. 4).

It is noteworthy that in the quadruped species, spasticity affected all four limbs: Cats stepped on the dorsal side rather than ventral side of their paws, due to enlarged forelimb flexor tone, suggestive of lead pipe paralysis (Fig. 2B). The hindlimbs frequently showed extensor spasms (Fig. 2A and B).

Signs of depression, analgesia, or sedation were absent in all animals, but some hyperesthesia and increased alertness were noticeable. Cats and monkeys showed signs of irritative behavior which might have been due to their motor and sensory deficits.

With time, the symptoms tended to subside in intensity in most animals. However, a marked tendency for irreversibility was noted. Incoordination as a result of hindlimb extensor spasms was evident for as long as 2 months after injection. Furthermore, in some animals, the syndrome increased in severity after a few days, resulting in quadriplegia.

The BOC derivative of phenylalanine produced the same symptoms when tried in 16 mice, 10 rats, and 5 cats, manifesting about the same potency. However, none of the other amino acids (tried each on six mice and six rats) showed any of the aforementioned motor deficits; these were either the free compounds of their N-carbobenzoxy derivatives, including phenylalanine (Table 1) even in doses up to 800 mg/kg. The acetyl derivative of phenylalanine and other amino acids were ineffective. L–dopa tried on a similar number of animals in doses up to 200 mg/kg did not antagonize the CBZPA–induced spastic symptoms, nor did glycine in doses of 400 mg/kg have any effect, despite its reported antagonism of spasticity induced by spinal cord ischemia.

DISCUSSION

The mechanism proposed to mediate clinical and some experimental models of spasticity is an autogenic facilitation of extensor flexes. This was presumed to result as a consequence of a specific de-efferentation of supra-spinal inhibitory pathways which normally exert regulatory influences on spinal motoneurons [3, 6–8, 28]. According to Granit *et al.* [18], when toxic extensors are activated

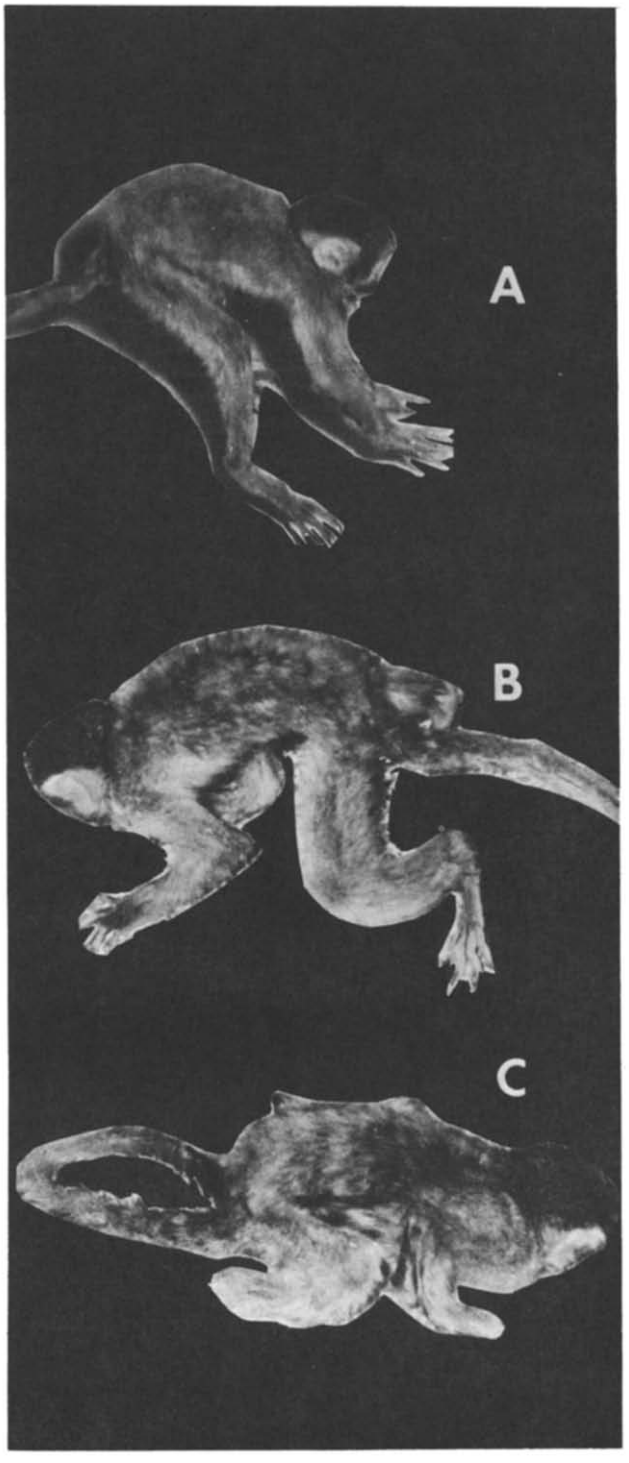

FIG. 4. Deficiency in antigravity responses shown in a squirrel monkey a few minutes after receiving a single injection of 100 mg/kg CBZPA manifested by the motor activity of the animal when dropped from a small height. (A) Control. (B) and (C) Four and ten minutes following the injection, respectively, the animal increasingly fails to break a fall from a small height, when flexor spasms replace the normal limb extension of the antigravity reaction.

during running movements, inhibition is generated at the same time, reaching a peak at the point where flexion begins. Decandia *et al.* [13] proposed that a process of disinhibition unfolds as well, and is responsible for an appropriately timed termination of the above-mentioned inhibition. If such a disinhibition starts too early, as may be the case in spasticity, the extensors do not come under inhibition at the time when flexion has to begin.

The question raised here is whether such a mechanism could explain the symptoms produced by N-carbobenzoxy-l-phenylalanine (CBZPA), since these symptoms are largely spastic in nature. In the monkey spastic symptoms manifested themselves during running. Forelimb flexor spasm appeared just as the flexing limb reached a point from which normally extension begins to take place. In cats, rats and mice, hindlimb extensor spasms appeared in the extending limb just as it reached a point from which flexion should begin. Hypertonicity was still another symptom typical of spasticity observed. It is of some interest that the fore- and hindlimbs were affected differently by CBZPA [33,35], especially in the phylogenically higher animals, as is the case also in clinical spasticity.

This difference in effects on the forelimbs and the hindlimbs is explainable on the basis of physiological and functional differences or differences in neurotransmitters mediating the reflexes of the fore- and hindlimbs, respectively [1].

The absence of overt symptoms in treated rats, cats, and monkeys at rest, the signs appearing during movement of the animals, is of some interest. A buildup of effect consequent to activity was suggested to relate to a defective balance in neurotransmitter substances [20]. However, other explanations are possible, such as dependency on sensory information from the limbs, which is characteristic also of γ-type spasticity. Involvement of the flexor reflex afferent system (FRA) is possible since it also manifests significant differences between forelimb and hindlimb spinal organization, and also shows dependency on state-in-space information [7, 8, 30].

Comparison may be drawn between the CBZPA-induced spastic symptoms and that occasionally observed in the hindlimbs of patients following spinal cord ischemia (Blum *et al.*, unpublished). This type of spasticity has been produced also by a temporary aortic occlusion [16,36], in which case it was suggested that interneuron damage was implicated [16, 32, 38]. However, this spasticity may be antagonized by the administration of glycine, presumably by a replenishment of the inhibitory neurotransmitter lost when the interneuron nerve endings are damaged [36]. In the present experiments, however, it was shown that glycine has no effect. The CBZPA-induced spastic symptoms are also of a greater complexity. It may be concluded, therefore, that in the CBZPA-induced spastic symptoms, glycine deficiency is not involved.

It is noteworthy that in phenylketonuria, symptoms of hyperreflexia, hypertonia and tremor appear in 70–75% of patients [14, 22, 23, 40]. Some of these symptoms are typical of spasticity, and were assumed to be extrapyramidal in origin [23].

Phenylalanine is the precursor of aromatic monoamine neurotransmitters involved in normal motor function [1,21]. Hassler *et al.* [20] have shown that disturbances in the aromatic monoamine neurotransmitters may lead to specific movement disorders. It is therefore possible that CBZPA may produce the above-mentioned symptoms by

virtue of its structural similarity to phenylalanine [5]. The involvement of the dopamine or norepinephrine production pathways became untenable, however, on account of the lack of effectiveness of l-dopa in antagonizing the CBZPA-induced spastic symptoms [25,39].

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