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THE MODE OF ACTION OF MYANESIN

BY

F. M. BERGER

From the Research Department, The British Drug Houses, Ltd Graham Street, City Road, London, N.1

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Berger and Bradley (1946, 1947) have shown that α: β-dihydroxy-γ-(2-methylphenoxy)-propane ("myanesin") produces muscular relaxation and paralysis in experimental animals. The effects of the drug are of particular interest because paralysing doses of myanesin do not cause arrest of respiration. In this respect myanesin differs from curare and similar muscle-relaxing agents, which do not produce paralysis without simultaneous respiratory depression or arrest. It was therefore of interest to examine in greater detail the mechanism by which the effects of myanesin are produced. This report describes certain aspects of the action of the drug on voluntary muscle, the myoneural junction, peripheral nerves, and the central nervous system.

Action on the isolated voluntary muscle

The action of myanesin was investigated on the isolated rectus abdominis of the frog (*R. temporaria*) during November and December. The muscle was suspended in oxygenated Ringer's solution and arranged for recording the contractions on a kymograph. Concentrations of drugs were expressed as final concentrations in contact with the muscle. Myanesin at 1 in 750 or higher dilutions did not cause any effects. Stronger solutions such as 1 in 500 produced a slow contracture, which began about 10 minutes after the addition of the drug and gradually increased until about 1 hour later the muscle had shortened by about one quarter of the maximum possible contraction. At this stage the muscle still responded to electrical stimulation.

The effect of myanesin on the contraction produced by acetylcholine was also investigated. Acetylcholine, 1:100,000, produced almost maximum contraction; after 2 similar contractions had been produced, myanesin or other drugs were added and allowed to remain in contact with the muscle for 10 minutes. The muscle was then washed and the response to acetylcholine retested at suitable intervals until contractions were obtained similar to those before application of the drug.

Table I gives the reduction of the contraction expressed as the percentage of the contraction before administration of the drug. Myanesin 1 in 1,000 reduced the response to acetylcholine to about one half and 1 in 750 to about one third. Stronger solutions of myanesin were not used because they affected the muscle itself. Tubocurarine chloride and procaine hydrochloride, when similarly examined, were found to exert a very much stronger effect than myanesin. The

TABLE I

THE ANTAGONISTIC EFFECT OF MYANESIN AND TUBOCURARINE ON CONTRACTIONS OF THE FROG'S RECTUS ABDOMINIS

Contractions were produced by acetylcholine 1 in 100,000

Drug	Dilution	Reduction of contraction as per cent of original contraction
Myanesin	1 : 2,000	25
	1 : 1,000	53
	1 : 750	68
Tubocurarine chloride	1 : 1,000,000	36
	1 : 750,000	46
	1 : 500,000	60
	1 : 100,000	93

concentrations of tubocurarine chloride, procaine, and myanesin causing approximately 50 per cent reduction in the response to acetylcholine were 1 in 700,000, 1 in 6,000, and 1 in 1,000 respectively.

The experiments show that myanesin in high dilution does not antagonize the action of acetylcholine on voluntary muscle. It is therefore likely that myanesin produces its effects by a mechanism different from that of curare. Although myanesin and procaine are local anaesthetics of almost equal potency, procaine antagonizes the effects of acetylcholine more strongly than myanesin. This observation suggests that the curare-like action of local anaesthetics is an independent property of such drugs and may have little relation to their paralysing effects on nerve endings or nerve trunks.

Effect on peripheral nerves

The action of myanesin on the motor nerve was examined on a simple muscle nerve preparation of the frog. The sciatic nerve was immersed in a solution of the drug. Excitability was tested at the cut end of the nerve at 1 minute intervals with galvanic current from an induction coil. The time after which contraction of the muscle was abolished was noted. The action on sensory nerves was examined by the plexus anaesthesia method in frogs and the intracutaneous weal method in guinea-pigs as described by Bülbring and Wajda (1945). Cocaine or procaine were used as standards of comparison. All drugs were used at three dose levels.

TABLE II

LOCAL ANAESTHETIC POTENCY OF MYANESIN EXPRESSED IN TERMS OF PROCAINE AND COCAINE

Method	Number of animals used with myanesin	Number of animals used with procaine	Potency as percentage of that of procaine	Number of animals used with cocaine	Potency as percentage of that of cocaine
Motor nerve (frog)	12	12	98	12	35
Sensory nerve (frog)	12	12	96	12	39
Intracutaneous weal (guinea-pig)	18	18	69	—	—

The results are summarized in Table II. Myanesin had about the same potency as procaine and one third that of cocaine when tested on the motor and sensory nerve of the frog. When examined by the intracutaneous weal method in guinea-pigs, myanesin had about two thirds of the activity of procaine. The relatively weak local anaesthetic action of myanesin suggests that the paralysing effect of the drug is not due to a direct action on peripheral nerves.

The curare-like action

It has been shown previously that myanesin in large and nearly lethal doses can produce paralysis of the muscle to indirect but not to direct stimulation. This effect may be due either to a curare-like action at the myoneural junction or to a more central paralysis of the nerve-endings (Berger and Bradley, 1946). Further experiments were carried out to ascertain the importance of this effect in myanesin paralysis.

(*a*) *Experiments on cats.*—Chloralosed or decerebrated cats were arranged for registration of contractions of the gastrocnemius muscle. The muscle was stimulated alternately directly and indirectly at 10 seconds intervals by single induction shocks. Intravenous injection of myanesin in doses up to 150 mg. did not effect the response to direct or indirect stimulation in any way. Tubocurarine chloride (0.5 mg.) abolished the response of the muscle to indirect stimulation but hardly influenced the response to direct stimulation. The experiments show that myanesin injected into cats in doses of 150 mg. does not possess curare-like action.

(*b*) *Experiments on mice.*—White mice weighing 18 to 22 grams were injected intraperitoneally with myanesin, cocaine, or procaine. After 10 minutes they were anaesthetized with ether and decerebrated. The sciatic nerve was then exposed in the gluteal region and cut. Fifteen and thirty minutes after injection of the drug, the peripheral end of the nerve was stimulated with faradic current of increasing voltage and the presence or absence of contraction of the muscles of the leg was noted.

Myanesin in doses of 300 mg. per kg. (i.e., about 50 per cent of the LD50) did not cause paralysis of the muscle to indirect stimulation. Larger doses such as 500 mg. per kg. (80 per cent of the LD50) made even the strongest stimulation of the nerve ineffective after 30 min., but did not significantly alter the threshold to direct stimulation. The disappearance of the response to indirect stimulation could be caused by a depression of neuromuscular transmission or by paralysis of the nerve. It was of interest to ascertain whether other local anaesthetics, such as cocaine or procaine, could paralyse motor nerves when administered in very large doses. Cocaine in doses as large as 100 mg. per kg. (LD75) did not abolish or impair the response to indirect stimulation. Similar results were obtained with procaine. It does not appear possible to produce paralysis of peripheral nerves by the systemic administration of local anaesthetics such as cocaine or procaine. It is therefore unlikely that paralysis to indirect stimulation produced by large doses of myanesin could be due to a paralysing action on the nerve, particularly as myanesin is a less potent local anaesthetic than cocaine. Myanesin may have an effect on the nerve because of a selective affinity of the

nerve tissue for the drug, but no evidence in favour of this assumption is available. It appears more likely that myanesin can block neuromuscular transmission when administered in very large doses. This curare-like effect does not play any part in the production of reversible muscular paralysis with smaller doses because under such conditions paralysis to indirect stimulation was never observed.

The myanesin–strychnine antagonism

It has been shown previously that myanesin can antagonize the actions of strychnine (Berger and Bradley, 1946). In those experiments the drugs were administered subcutaneously in 2.5 per cent gum acacia solution. To measure the antagonistic effect more accurately a series of experiments was carried out in which the drugs were administered intravenously. With this mode of administration, variations due to differences in the speed of absorption were eliminated.

White mice weighing 18 to 22 grams were used. Injections were made into the tail vein at a rate of 0.3 c.c. per minute. The convulsant and myanesin were injected together in a volume of 0.4 c.c. per 20 grams body weight.

The minimal lethal doses (MLD), approximately equal to the LD80, of strychnine sulphate and leptazol were 0.43 and 100 mg. per kg. respectively. The median lethal dose (LD50) of myanesin was 320 mg. per kg.

Mice were injected with single minimal lethal doses of strychnine or leptazol, or multiples thereof. Myanesin was administered simultaneously and, for each dose of the convulsant, doses of myanesin were found which protected some of the animals from death. From these values the dose of myanesin protecting 50 per cent of animals was found graphically by plotting the probits of the percentage mortality against the log doses.

TABLE III

STRYCHNINE–MYANESIN ANTAGONISM AFTER SIMULTANEOUS INTRAVENOUS ADMINISTRATION TO MICE

Strychnine dose as multiple of MLD	Myanesin		Ratio to number injected of number		Dose of myanesin protecting 50% of mice mg./kg.
	mg./kg.	fraction of LD50	convulsed	died	
1	—	—	35/35	30/35	
1	40	1/8	0/10	0/10	12
1	20	1/16	2/10	2/10	
1	10	1/32	9/10	6/10	
2	80	1/4	0/20	0/20	
2	40	1/8	17/20	7/20	34
2	20	1/16	10/10	9/10	
3	320	1	0/10	0/10	
3	160	1/2	6/10	2/10	80
3	80	1/4	10/10	5/10	
4	320	1	7/20	12/20	
4	160	1/2	20/20	10/20	160
4	80	1/4	10/10	10/10	

The antagonistic action of myanesin against leptazol was relatively weak. About one quarter of the LD50 of myanesin protected 50 per cent of the animals against 1 MLD of leptazol. The effect of myanesin in maintaining life was greater than its power to prevent the occurrence of convulsions. Protection against 2 MLD of leptazol could not be obtained even when administered together with one LD50 dose of myanesin.

The antagonistic action of myanesin to the effects of strychnine was well marked. Animals could be protected from the effects of one MLD dose of strychnine by as little as one thirtieth of the LD50 of myanesin, and proportionally larger doses were able to antagonize larger doses of strychnine (Table III).

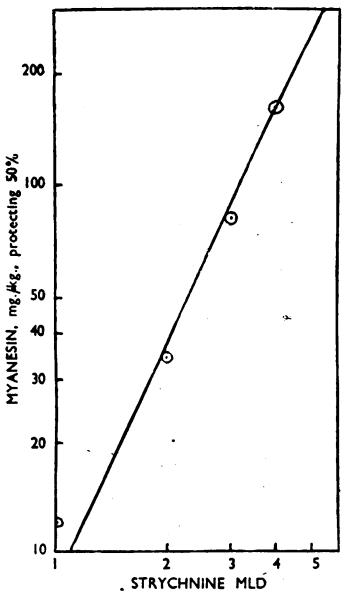

FIG. 1.—Antagonism of myanesin and strychnine. Abscissae: Dose of strychnine as multiple of MLD. Ordinates: Dose of myanesin protecting 50 per cent of animals.

When the median protective dose of myanesin was plotted against the dose of strychnine, expressed in terms of MLD, both values being plotted on a logarithmic scale, an approximately straight line was obtained (Fig. 1).

In suitable dosage myanesin antagonized all the effects of strychnine, and animals to which balanced mixtures of the two drugs had been administered appeared quite normal. The myanesin–strychnine antagonism was more complete than the hexobarbitone–strychnine antagonism ; in the latter it was not possible to find a balanced mixture, because doses of hexobarbitone which protected mice from convulsions and death caused a depression of greater duration and intensity in the presence of strychnine than in its absence.

Strychnine causes an increase of the reflex irritability of the spinal cord which may result in simultaneous contraction of all skeletal muscles if sufficiently large doses are given. Myanesin selectively antagonized the effects of strychnine in doses which by themselves did not produce any effects. It may therefore be inferred that myanesin decreased reflex hyperexcitability and prevented the passage of abnormal excitatory impulses through the reflex arcs.

Effect on tetanus

The effect of myanesin on experimental tetanus was examined in white mice. Ten micrograms of crude tetanus toxin were injected into the neighbourhood of the sciatic nerve high in the thigh. Local tetanus was apparent about 12 hours later and all animals died of generalized tetanus in about 3 days. Myanesin was administered intraperitoneally or subcutaneously at various times after tetanic spasms became apparent. In doses of

150 to 200 mg. per kg. it completely abolished the spasm in all stages of the disease. The board-like rigidity of the extremities gave place to complete flaccidity a few minutes after injection of the drug. Myanesin in these doses did not cause depression of respiration or any other untoward effects and poisoned animals under the influence of the drug were indistinguishable from control animals to which myanesin only had been given. The effect of the drug lasted for about 30 min. and was followed by a gradual reappearance of tetanus. Repeated doses of myanesin caused similar effects and regularly released the spasm. The impression was gained that the life of the animal could have been saved by continuous or frequently repeated administration of the drug.

The action of tetanus toxin on the central nervous system is similar to that of strychnine. The fact that myanesin can re-establish reciprocal innervation in conditions produced by these two different agents gives rise to the hope that it may also influence pathological excitatory innervation caused by degenerative processes of the central nervous system.

Effect on knee jerk

The effect of myanesin on the knee jerk was studied in cats. The animals were anaesthetized with chloralose intravenously and arranged for recording of the knee jerk as described by Schweitzer and Wright (1937). The limb was allowed to hang freely in order to facilitate observation of alteration in muscle tone. The jerk as a rule was elicited every 10 seconds.

Myanesin did not abolish the knee jerk of healthy cats. Intravenous doses ranging from 20 to 150 mg. per cat either did not cause any alteration or somewhat reduced the height of the myographic record (Fig. 2); the flexion at the knee joint was usually increased,

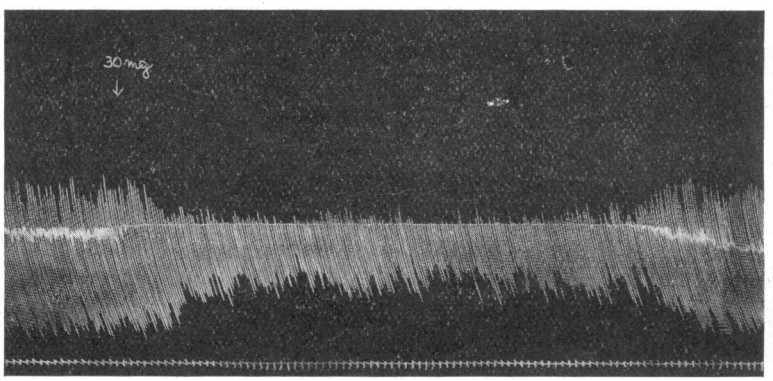

FIG. 2.—Cat 2.8 kg. Chloralose (0.08 g. per kg.). Record of knee jerk. Time intervals 30 seconds. At arrow 30 mg. myanesin slowly injected intravenously.

signifying a decrease in the tonus in the muscles, but complete inhibition of the jerk was not observed.

Some of the cats used in the experiments showed a very lively reflex followed by clonus. Exaggerated reflex excitability and tremors were also observed in certain animals under light chloralose anaesthesia (0.05 g. chloralose per kg.). Myanesin had a definite

effect on the knee jerk of these animals (Fig. 3A and B and Fig. 4). It inhibited clonus and tremors, abolished the irregular responses to patellar stimulation and reduced the knee jerk to its usual size. These effects could be produced with small doses of myanesin (20 to 30 mg. per 3 kg. cat). The effect set in almost immediately and lasted for about 20 minutes and sometimes longer.

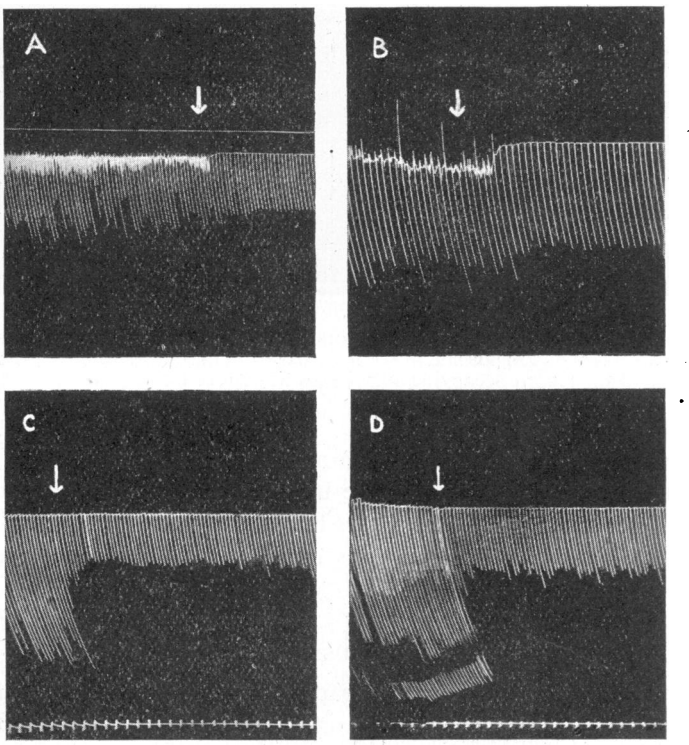

FIG. 3.—Records of knee jerk in cats weighing 2.8–3.1 kg. Jerk elicited every 10 seconds. Drugs injected intravenously. A. Chloralose 0.05 g. per kg., myanesin 50 mg. B. Chloralose 0.06 g. per kg., myanesin 150 mg. C. Chloralose 0.06 g. per kg., strychnine 0.2 mg., myanesin 50 mg. D. Chloralose 0.05 g. per kg., strychnine 0.4 mg., myanesin 100 mg.

The effect of myanesin on the experimentally increased knee jerk was also studied. Cats were injected with doses of strychnine insufficient to cause convulsions but producing exaggerated reflex activity. Myanesin in small doses promptly counteracted this increased reflex excitability and caused an immediate reduction of the reflex to the level present before administration of strychnine. (Fig. 3C and D.)

The effects of myanesin on the knee jerk of rabbits anaesthetized with urethane were also examined; they were similar to those observed in cats. Myanesin, 100 mg. per rabbit weighing about 3 kg., caused a decrease in the size of a few jerks immediately following the injection. Smaller doses did not influence the size of the jerk but abolished tremors, clonus and spontaneous movements of the leg.

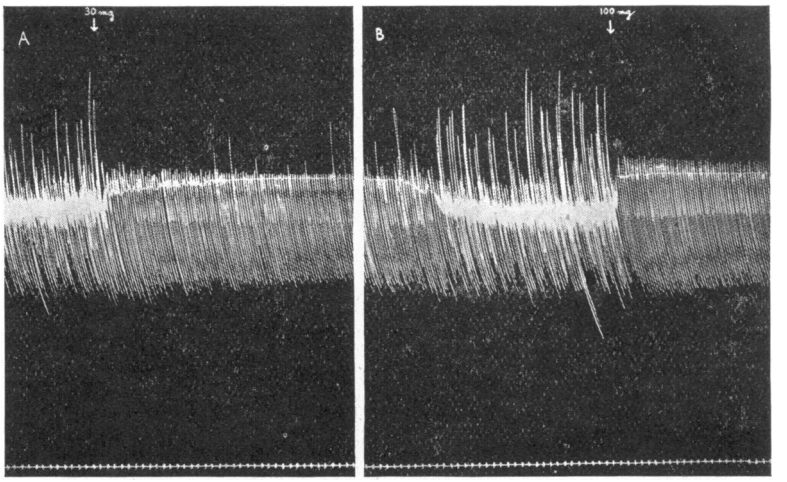

FIG. 4.—Record of knee jerk. Cat 3.4 kg. Chloralose 0.08 g. per kg. Time interval 30 seconds. At first arrow 30 mg. myanesin, at second arrow 100 mg. myanesin i.v. Between A and B a piece of the record occupying 40 minutes was cut out.

The experiments show that although myanesin has little effect on the normal knee jerk of the cat, it is very effective in reducing an exaggerated reflex to its normal size. Myanesin in suitable doses may therefore exert an inhibiting action on pathologically exaggerated functions of the central nervous system without influencing normal reflexes.

DISCUSSION

The experiments reported in this paper show that the effects produced by myanesin are due to its peculiar action on the spinal cord. Myanesin selectively depresses hyperexcitability of spinal reflexes, but hardly influences the normal reflex actions mediated through the cord. Symptoms of hyperactivity, whether due to the action of poisons or to light anaesthesia, can be inhibited with smaller doses of myanesin than those required for the depression of normal physiological functions. Strychnine convulsions in mice can be counteracted with as little as 20 mg. per kg. intravenously, but 150 mg. per kg. or more, administered by the same route, are required to paralyse the animal. In cats 20 mg. abolished tremors and clonus, but 150 mg. did not abolish the knee jerk. The relation between the dose required to bring hyperfunction back to normal and that causing depression of normal function was approximately the same with mice, rabbits, and cats.

The depressant action of myanesin on the peripheral nerves (the local anaesthetic action) is too weak to play any part in the effects produced by systemic administration of myanesin. In concentrations which can be achieved after systemic administration, myanesin does not exert any direct relaxing action on

muscles and does not block the action of acetylcholine on them. The curare-like action obtained with large doses is of toxicological interest only and never becomes apparent with doses from which animals recover. It is incorrect to call myanesin a curarizing agent, because doses causing relaxation during anaesthesia do not influence neuromuscular transmission, but produce relaxation by a depressant action in the spinal cord.

Muscular relaxation produced by myanesin differs from that produced by curare not only in that the drugs act on different structures, but also in the order and degree in which various groups of muscles are affected. With curare, muscles with cranial innervation are affected first; the peripheral and intercostal muscles are paralysed next, and with complete curarization the diaphragm is affected to almost the same extent as other muscles. With myanesin the muscles of the posterior half of the body are affected first, next the peripheral and intercostal muscles, then the cranial muscles; the diaphragm is affected last and there is a distinct margin between doses causing muscular paralysis and those causing arrest of respiration.

Both curare and myanesin have been used for the production of muscular relaxation during anaesthesia (Mallinson, 1947). Curare is the agent of choice for complete suppression of respiration, such as is required during certain operations on the lungs. Myanesin, on the other hand, appears to be more useful for the production of muscular relaxation when suppression of respiration is not desired. Myanesin should never be used for the production of respiratory arrest, because the doses required for this purpose are large and affect the heart and blood pressure.

Occasionally when an attempt was made to produce respiratory arrest in an already paralysed rabbit by rapid intravenous injection of myanesin, a general rigidity without arrest of respiration developed. The rigidity, which in appearance was similar to decerebrate rigidity, lasted for about 1 min. and was followed by profound muscular relaxation from which the animals recovered. The cause of this symptom is not understood. It may be due to a direct action on the muscle. It was observed only in certain rabbits after rapid intravenous injection of concentrated solutions. It was not seen in anaesthetized animals. Other species of animals, as well as most rabbits, showed respiratory arrest if administration was continued after complete paralysis was obtained.

The rigidity observed after rapid intravenous injection to certain non-anaesthetized rabbits is the only sign of stimulation which has been observed with myanesin. With this exception myanesin caused pure depression in all species which were examined and at all dose levels. Crystalline *d*-tubocurarine chloride, on the other hand, may cause conspicuous signs of central stimulation such as trembling, hyperexcitability to stimuli, muscle twitching, and convulsions. These symptoms are particularly well marked in mice and rats, but can also be observed in other species (Cohnberg, 1946).

West (1935) observed that certain samples of curare removed the violent spasm of parathyroid tetany in dogs without paralysing the animal. This selective removal of pathological rigidities without apparent diminution of voluntary power was termed lissive action by West. Pure crystalline tubocurarine chloride possessed no trace of lissive power in dogs.

Experiments reported in this paper indicate that myanesin possesses a lissive action. It should, therefore, prove useful in the treatment of spastic paralysis, hypertonic states, and tremors. It may be expected that myanesin will be effective in these conditions in doses which do not affect consciousness, do not diminish muscular power, and do not cause side effects. Similar results may also be expected after oral administration because myanesin is well absorbed from the intestinal tract.

SUMMARY

1. Myanesin in high dilution had no direct action on the rectus abdominis of the frog and did not block the action of acetylcholine on this muscle.

2. The local action of myanesin on peripheral nerves was similar to that of procaine.

3. In doses causing reversible paralysis, myanesin did not possess a curare-like action, but toxic doses had a blocking effect on the myoneural junction.

4. Myanesin had but little effect on the normal knee jerk. An exaggerated knee jerk due to light anaesthesia, strychnine, or unknown causes was promptly reduced to the usual size.

5. Myanesin in small doses antagonized all the effects of strychnine and relieved tetanic spasm.

6. Myanesin had a selective depressant action on the spinal cord. In doses which had little effect on voluntary power it restored deranged reciprocal innervation to normal and counteracted symptoms caused by a release from inhibitions as observed during light anaesthesia. The powerful effect of myanesin on tremors, increased reflex excitability, and similar symptoms suggests that it may be useful in the treatment of spastic and hypertonic conditions.

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