ACTION OF MEPROBAMATE ON SPINAL MONOSYNAPTIC REFLEXES AND ON INHIBITORY PATHWAYS

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ABSTRACT

Meprobamate was administered intravenously to spinal cats, in cumulative doses of 30 to 40 mg./kg. each. Initial doses may have a variable action on monosynaptic reflexes. At times some reflexes are depressed, while others are enhanced or unaffected. When dose levels of 100 mg./kg. or higher are reached, monosynaptic reflexes, both flexor and extensor, are depressed. Monosynaptic reflexes can be strongly depressed by meprobamate, their input-output relations often remaining unchanged. In such cases there is thus no change in the spatial summation requirements of those motoneurons remaining in the excitable zone. Inhibitory pathways, both direct and disynaptic, are highly resistant to the action of meprobamate. The drug does not distinguish between the direct and disynaptic pathways. It is suggested that meprobamate acts as a general depressant of excitatory synaptic transmission.

INTRODUCTION

Meprobamate[1] (2-methyl-2-n-propyl-1,3-propanediol dicarbamate) has been described as a long lasting muscle relaxant and sedative drug, that also exerts a blocking action on spinal interneurons (2). Some have compared the action of this drug with that of mephenesin and both of these drugs have been described as interneuron depressants (1, 2). On the other hand, others have classified meprobamate as a "barbiturate-like drug with some CNS stimulant properties that may simulate those of trimethadione" (13).

Meprobamate, like mephenesin, depresses some spinal polysnyaptic reflexes, mephenesin, however, being the more potent of the two drugs (13). In the case of mephenesin, monosynaptic reflexes have been found to be much more resistant to depression than are polysynaptic reflexes. At times, doses of mephenesin sufficient to depress polysynaptic pathways enhance monosynaptic responses, probably due to a lessening of background inhibitory internuncial activity (4, 15). With higher dose levels, however, monosynaptic reflexes are depressed (9, 15, 17), and more sensitive tests utilizing heteronymous monosynaptic transmission give evidence of depression with lower doses (11). Be-

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cause of the differential susceptibility of polysnyaptic and monosynaptic reflexes it has been claimed that the action of mephenesin is fairly specific to internuncial cells (7). An alternative explanation is that the drug acts at all synapses, polysynaptic pathways being more vulnerable because of the cumulative action on the larger number of junctions involved (17).

The following experiments present a study of the action of meprobamate on several types of spinal pathways, in particular monosynaptic excitatory pathways and the direct and disynaptic inhibitory pathways.

Methods

Decapitate cats were used in all experiments. Laminectomy was performed in the usual manner and the appropriate ventral roots were cut. A number of peripheral muscle nerves were prepared for stimulation. The spinal cord was covered by a pool of warm mineral oil, the temperature of which was kept steady throughout the experiment.

Reflexes were evoked by stimulation of selected muscle nerves and recorded in cut ventral roots. Afferent volleys were recorded by an electrode at the dorsal root-cord junction. The size of the afferent volleys was determined by the peak-to-peak amplitude of the triphasic response.

Meprobamate was dissolved in warm mammalian Ringer's solution, at a concentration of 15 mg./cc. Intravenous injections were made over a period of several minutes, and 20 minutes were allowed to elapse before determinations were begun. The drug was administered in cumulative doses of 30 to 40 mg./kg. each; successive injections were given at regular intervals, usually 40 minutes.

RESULTS

1. Action of Meprobamate on Monosynaptic Reflexes

Injection of meprobamate in cumulative doses of 30 to 40 mg./kg. gradually depresses all monosynaptic reflexes. A typical series of results is presented in Fig. 1, which shows from a series of seven preparations the effect of the drug on the amplitude of gastrocnemius monosynaptic reflexes. Initial doses had little effect, and depression was present unequivocally only when levels of the order of 100 mg./kg. were reached. For this set of experiments the 20 per cent depression point was obtained with doses ranging from 65 to 155 mg./kg., with a geometric mean of 107. To produce 50 per cent depression, doses ranging from 130 to 195, with a geometric mean of 165, had to be given. While the results illustrated in Fig. 1 are fairly representative, reflex depression was obtained with lower doses in some experiments. Out of 25 various monosynaptic reflexes studied in 12 cats, 7 were depressed by doses under 60 mg./kg. Another factor, possibly the condition of the preparation, seems to be important in determining the minimal dose at which the drug becomes effective.

In several preparations the initial doses had different effects on the various reflexes simultaneously under investigation; some of these were reflexes evoked

by impulses in afferent nerves to antagonistic muscles. At times one reflex was depressed, while another was enhanced or unaffected. The weaker reflex was not necessarily depressed first. With higher dose levels depression of all monosynaptic reflexes, both flexor and extensor, was regularly obtained.

The input-output curve for the monosynaptic reflex, which relates magnitude of reflex discharge to that of the causal afferent volley, is a useful tool for the study of spatial summation in reflex transmission (8, 12, 14). Some use has been made of this type of curve in the study of drug action on reflex phenomena, in particular on post-tetanic potentiation (6). The effect of meprobamate on input-

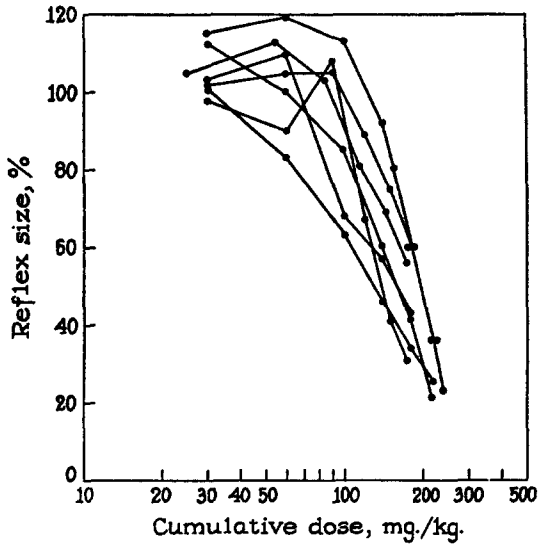

FIG. 1. Effect of cumulative doses of meprobamate on gastrocnemius monosynaptic reflexes in seven animals. In each experiment the control value is taken as 100 per cent. Each point represents the average of approximately 15 measurements.

output curves has been investigated to see if such an approach might add to the information obtained by means of tests utilizing maximal shocks only.

Afferent volley size was measured throughout the course of the experiments, and it could therefore be shown whether any reductions in reflex size were due to a peripheral action of the drug. The size of the spike fluctuated during some experiments, but did not change in a consistent manner. Any observed reflex changes were therefore not due to the action of the drug on afferent nerves.

In the experiment shown in Fig. 2, the absolute size of a monosynaptic reflex, in this case the reflex evoked by stimulation of the nerve to flexor longus digitorum, was reduced to 44 per cent of the control size by cumulative doses of meprobamate totalling 220 mg./kg. The input-output curve, however, re-

mained unchanged: a given per cent of afferent input still activated the same per cent of those neurons in the pool that could be made to respond by a maximal shock. In some experiments, reduction in the size of one reflex was accompanied by a shift of the input-output curve to the right, indicating an increase in the spatial summation requirements of the pathway studied, whereas another reflex, recorded simultaneously in the same preparation, could be depressed without any shift of the curve. The type of result illustrated in Fig. 2 and Fig. 4 A has been observed frequently, showing that pronounced depression of reflex

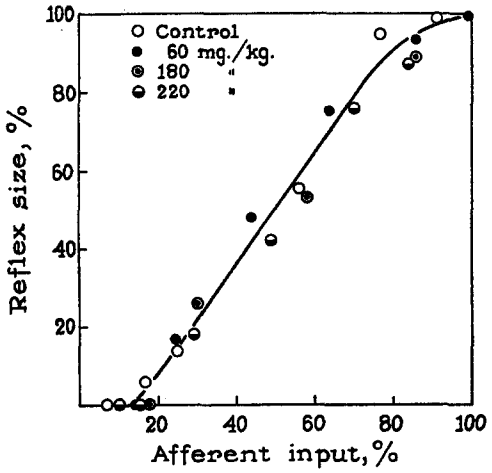

FIG. 2. Input-output curve for monosynaptic reflex of flexor longus digitorum. At each dose level the mean response obtained at maximal input is taken as 100 per cent. At a dose level of 60 mg./kg. the reflex had been reduced to 92 per cent of control size; at 180 mg./kg., to 54 per cent; at 220 mg./kg., to 44 per cent.

amplitude can be obtained without any change in the input-output relations of the response.

2. Action of Meprobamate on Spinal Inhibitory Pathways

The action of meprobamate on direct inhibition and disynaptic inhibition has been studied in seven experiments; in four of these the effects on the direct and disynaptic pathways were compared in the same preparation. With the exception of one experiment in which the deep peroneal monosynaptic reflex served as the test response, the gastrocnemius monosynaptic reflex was used as the test response throughout. Direct inhibition of this test reflex was obtained by volleys in the deep peroneal nerve; disynaptic inhibition resulted from afferent volleys in the nerve to flexor longus digitorum (10). The shock interval was routinely set for maximum inhibition.

Fig. 3 illustrates the typical result obtained in all but one experiment. Fig. 3 A shows that meprobamate depresses the uninhibited monosynaptic test response. The size of the inhibited response is also reduced, probably due to the depressant action of meprobamate on the test system. Low doses may increase the size of the test reflex. This increase is frequently accompanied by a parallel increase in the size of the inhibited test response. Specific increase in the size of the inhibited test reflex, occurring in the absence of change in the uninhibited test reflex, usually has not been seen.

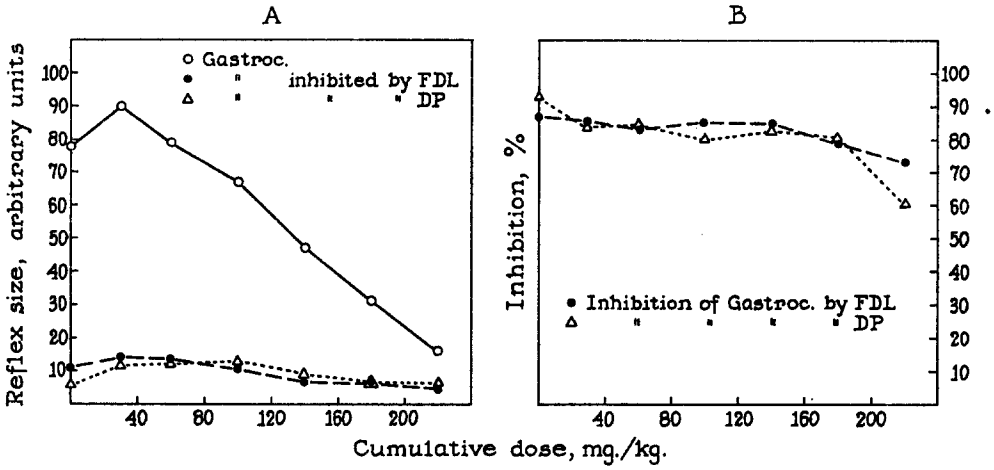

FIG. 3. Action of meprobamate on direct and disynaptic inhibition. A, effect of drug on the absolute size of test reflex (gastrocnemius), and on the size of the test reflex when inhibited by direct (triangles) or disynaptic (dots) inhibitory action. B, same experiment; effect of drug on per cent direct inhibition (triangles) and disynaptic inhibition (dots).

The lack of effect of meprobamate on the efficacy of inhibitory pathways is demonstrated in Fig. 3 B, which shows the degree of inhibition, measured in per cent, at different levels of meprobamate. Except with the largest doses there is little change in the per cent inhibition: throughout most of the experiment the inhibitory volley is able to prevent the firing of approximately the same percentage of those motoneurons still in the excitable zone. The gradual decrease in the number of motoneurons that can be activated by a maximal shock can account for the downward drift sometimes seen in the measurements of per cent inhibition, as the decrease in size of the inhibited test reflex may be relatively smaller than is the reduction of uninhibited responses. The increase in the height of the uninhibited and inhibited test reflexes which sometimes occurs in the beginning of the experiment also can result in fluctuation of per cent inhibition.

Experiments such as that illustrated in Fig. 3 also show that there is no difference in the action of meprobamate on the direct and disynaptic inhibitory pathways.

The effect of meprobamate on the relation between afferent input and degree of inhibition has been studied in some experiments with the result exemplified in Fig. 4. The gastrocnemius reflex served as test response, direct inhibition being obtained by stimulation of the deep peroneal nerve; the monosynaptic reflex resulting from stimulation of the deep peroneal nerve was also monitored.

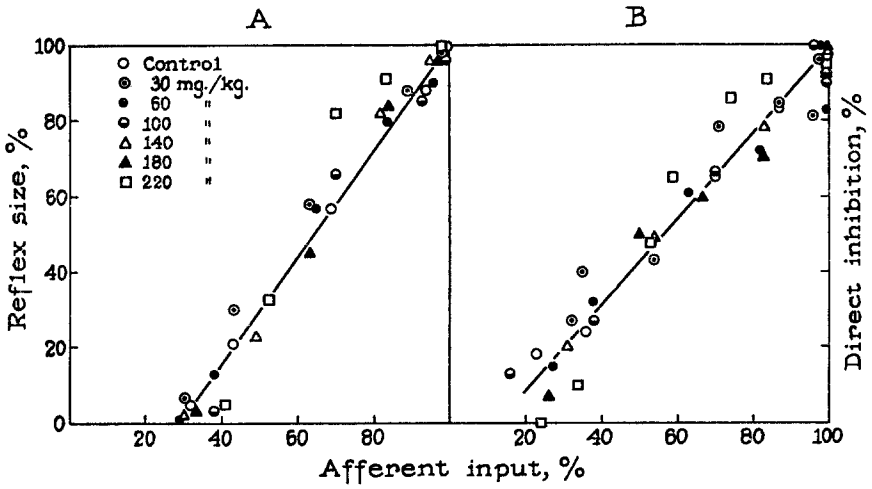

FIG. 4. Input-output curve for monosynaptic transmission and relation between afferent input and degree of direct inhibition, measured in the same experiment. A, input-output curve for deep peroneal monosynaptic reflex. At 30 mg./kg. reflex size was 115 per cent of control; at 60 mg./kg., 112 per cent; at 100 mg./kg., 104 per cent; at 140 mg./kg., 91 per cent; at 180 mg./kg., 84 per cent; at 220 mg./kg., 71 per cent. B, relation between degree of direct inhibition of gastrocnemius reflex and size of afferent volleys in the deep peroneal nerve.

By the time that a dose level of 220 mg./kg. had been reached, the gastrocnemius test response had been reduced to 25 per cent of control size, and the monosynaptic reflex evoked by stimulation of the deep peroneal nerve had been decreased to 71 per cent of control size. The degree of inhibition fluctuated somewhat, but was 77 per cent both at the beginning and the end of the experiment. In this experiment, in which both the reflex and the inhibition caused by volleys in the deep peroneal nerve were recorded, neither the input-output curve of deep peroneal monosynaptic reflex discharge (Fig. 4 A) nor the relation between afferent input and degree of direct inhibition (Fig. 4 B) was modified by meprobamate. This and two other experiments utilizing the rela-

tion between afferent input and degree of direct and disynaptic inhibition have shown that this relation does not seem to be specifically modified by meprobamate.

DISCUSSION

The experiments described in the first section of the results show that meprobamate acts as a depressant of monosynaptic reflexes in the cat spinal cord. The variable effects obtained on monosynaptic reflex amplitude with doses of 30 to 40 mg./kg. suggest that, as is the case with mephenesin (4, 15), the initial action of meprobamate is largely an indirect one; it results from a change in the background activity of internuncial pools, which, because of the large number of synapses they contain, are more susceptible to the action of the drug than is the monosynaptic arc. As dose levels of 100 mg./kg. and higher are reached, the depression of monosynaptic reflexes becomes evident. The fact that antagonistic flexor and extensor responses are both strongly depressed in the same preparation indicates that the effect is not only an indirect one, but also a direct one on some part of the monosynaptic pathway.

Input-output curves make it possible to study changes in the spatial summation requirements of monosynaptic reflexes. It is obvious from Figs. 2 and 4 A that meprobamate can leave the input-output relations unchanged while it decreases the amplitude of the reflex. Of particular interest is the fact that the reduction in reflex size is not necessarily accompanied by a shift of the origin of the input-output curve to the right. The point of origin of the curve indicates the least amount of spatial summation required to evoke a visible reflex discharge. If the drug acted by blocking the activity of presnyaptic terminals it would be expected that the minimal input level necessary to secure motoneuron discharge would be raised, unless the assumption is made that the terminals of the higher threshold afferent fibers are blocked selectively. The origin of the curve would correspondingly be shifted to the right. Therefore, the frequent absence of such a shift indicates that meprobamate does not block transmission in presynaptic structures.

Non-specific change in excitability, such as is brought about by change in body temperature (8), influences the magnitude of reflex discharge to any given size of afferent volley without affecting the form of the input-output relation. The common action of meprobamate in decreasing excitability without causing a shift in the input-output curve provides another example of this phenomenon, and as a result stands in contrast to the results of others who have described shifts in the input-output curve due to variation in depth of anesthesia (14), to post-tetanic potentiation (6, 14) or to temperature change (6). Change in the intensity of convergent excitatory synaptic activity does alter the input-output relation of a monosynaptic reflex (8). The occasional result of meprobamate injection, namely a shift of the origin of the input-output curve to the right,

may, at least in part, be due to removal of convergent facilitatory background.

The present experiments have failed to reveal any clear action of meprobamate on direct and disynaptic inhibition, other than an occasional decrease when high dosage levels are reached, and when there is, concurrently, a strong depression of the monosynaptic test reflexes.

Inhibition of the knee jerk by volleys in the ipsilateral sciatic nerve has been reported to be resistant to the action of mephenesin (7), and more recently (1) to be susceptible to the action of both mephenesin and meprobamate. The pathway for this inhibitory action would be disynaptic and polysynaptic (10), and direct if stimulation includes the hamstring nerves. The present experimental result is not necessarily in conflict with these observations on the knee jerk for the inhibitory pathways employed differed. Those pathways here studied do not include the polysynaptic pathways of flexor reflex action that must contribute heavily to inhibition of the knee jerk by sciatic nerve stimulation, and which may be more susceptible to meprobamate than are the direct and disynaptic paths.

The finding that inhibitory paths are seemingly less sensitive to meprobamate than is the monosynaptic reflex path points out a difference in the properties of inhibitory and excitatory junctions; it is in line with the fact that inhibitory pathways are less sensitive to other forms of depression than is the monosynaptic reflex. Direct inhibition is insensitive to low frequency depression (3, 16). Disynaptic inhibition is sometimes subject to low frequency depression, but is much less affected than is monosynaptic transmission (16).

Meprobamate, like mephenesin, has been classified as an "interneuronal blocking" agent, mainly because of its pronounced action on the ipsilateral flexor reflex (2). However, one finds that it does act upon the monosynaptic reflex path and conversely that the disynaptic inhibitory path, that contains one internuncial neuron, or more (5), in series, is relatively insensitive. From this it would seem more likely that meprobamate acts as a general depressant of excitatory synaptic transmission, with the degree of effect on any given pathway related to the number of relays it contains and the "power" of the individual relays.

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