

Recurrent Conditioning in the Cat Spinal Cord

Differential effect of meprobamate on recurrent facilitation and inhibition

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ABSTRACT The action of cumulative doses of meprobamate on antidromic conditioning has been studied in spinal cats. Recurrent facilitation is greatly reduced or completely abolished by total doses ranging from 210 to 400 mg./kg. The depth of recurrent inhibition is not affected in a consistent manner by meprobamate, but the duration of inhibition is markedly increased in all experiments. This differential action of meprobamate on facilitation and inhibition can be utilized to study conditioning effects consisting of combined inhibition and facilitation. If conditioning starts with an inhibitory phase, variable in duration, followed by facilitation, meprobamate depresses the facilitation and reveals an extended inhibitory curve. Facilitation, however, is not always accompanied by inhibition, since in some cases facilitation is depressed and no inhibition is uncovered. The results of these experiments are discussed in relation to the various types of conditioning that have been produced by antidromic stimulation.

INTRODUCTION

Recurrent inhibition and facilitation in the cat spinal cord have been known since the work of Renshaw (1941). Subsequent work has indicated that the inhibition is brought about by means of a pathway that includes the cholinergic motor axon collaterals and the Renshaw cells, which latter exert an inhibitory action on motoneurons (Eccles, Fatt, and Koketsu, 1954). Lately the recurrent facilitatory path also has been shown to include a cholinergic link, and the suggestion has been made that this facilitation is initiated by collaterals of the large motor fibers (Wilson, 1958*a*; 1959).

Renshaw (1941) observed that facilitation was usually preceded by inhibition. Mixed conditioning effects have been described by others. Lloyd (1951) demonstrated an inhibition which reverses to facilitation at a conditioning-test interval of about 45 msec. when monosynaptic reflexes in a ventral rootlet or muscle nerve were conditioned by antidromic stimulation

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of a neighboring rootlet, or of the appropriate muscle nerve. Granit, Pascoe, and Steg (1957) observed that antidromic conditioning of tonic motoneurons, whose activity was recorded in fine ventral root filaments, could lead to inhibition followed by an excitatory phase; they attributed this excitatory action to rebound. Mixed effects were observed by Wilson (1959), who also obtained facilitation that was not preceded by inhibition.

Meprobamate has been shown to depress the ipsilateral flexor reflex (Berger, 1954), the knee jerk (Abdulian, Martin, and Unna, 1957), and monosynaptic reflexes evoked by stimulation of muscle nerves (Wilson, 1958*b*). Abdulian *et al.* (1957) indicate that meprobamate depresses the inhibition of the knee jerk evoked by ipsilateral sciatic nerve stimulation. Wilson (1958*b*) found that both direct and disynaptic inhibition were highly resistant to doses of meprobamate that depressed monosynaptic reflex transmission. In view of the seeming difference between the actions of the drug on excitatory and inhibitory pathways shown in this latter paper a study has been made of the effect of meprobamate on recurrent facilitation, recurrent inhibition, and mixed inhibitory and facilitatory effects.

Methods

Decapitate cats were used in all experiments. Dorsal roots were cut from L 4 to S 3. Monosynaptic reflexes were elicited by stimulating the appropriate dorsal roots, and recorded in cut peripheral nerves. The conditioning stimuli were applied to peripheral nerves and the antidromic impulses entered the cord through the intact ventral roots.

Meprobamate¹ 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, usually 40 mg./kg. each; successive injections were given at intervals of approximately 40 minutes.

RESULTS

1. Effect of Meprobamate on Recurrent Facilitation

Meprobamate, administered in cumulative doses of approximately 40 mg./kg. each, exerts a depressant action on recurrent facilitation. It has been previously pointed out (Renshaw, 1941; Wilson, 1959) that this facilitation is often preceded by a variable amount of inhibition. In a series of five experiments (summarized in Table I) in which facilitation was preceded by only a small inhibitory component, or no inhibition at all, facilitation was reduced

¹ Meprobamate was kindly supplied by the Merck Institute for Therapeutic Research, West Point, Pennsylvania.

or completely abolished by cumulative doses ranging from 210 to 400 mg./kg. Doses of this magnitude usually reduce the size of the monosynaptic reflexes used for testing, but reduction in facilitation has been obtained

TABLE I
ACTION OF MEPROBAMATE ON RECURRENT FACILITATION

Experiment	Total dose	Reflex size		Facilitation in absolute units		Facilitation	
		Control	At end of exp.	Control	At end	Control	At end
1	210	31	28	14	2	<i>per cent</i> 42	<i>per cent</i> 10
2	260	47	50	19	0	37	0
3	290	55	42	7	2	14	<5
4	400	60	45	11	2	20	4
7	240	50	31	7	2P	14	<4

In all experiments the test reflex was in the deep peroneal nerve or one of its branches (usually tibialis anterior) and facilitation was evoked by antidromic stimulation of the nerve to triceps surae. Reflex sizes are given in arbitrary units. In Experiments 3 and 7 the action of the drug increased the magnitude of an inhibitory component which was very small in the control state (see section 3).

without simultaneous depression of the unconditioned test response. Fig. 1 shows the effect of meprobamate on facilitation of a reflex in the nerve to tibialis anterior brought about by an antidromic stimulus to the nerves of

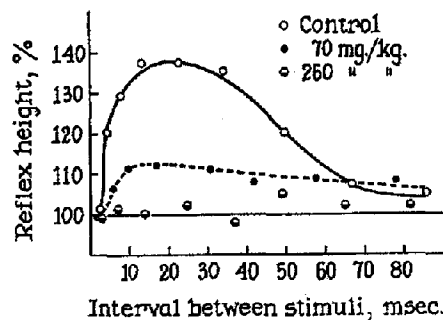


FIGURE 1. Effect of meprobamate on recurrent facilitation. The monosynaptic reflex in the nerve to tibialis anterior was facilitated by antidromic stimulation of the nerves to triceps surae. At 70 mg./kg. the test reflex in isolation was 130 per cent of the pre-drug control; at 260 mg./kg., 107 per cent. In this and in succeeding figures each point represents the average of 15 to 20 measurements.

triceps surae. In the experiments shown, the test reflex was increased in size following the initial injection of meprobamate, and was just back to control level when the total dose of 260 mg./kg. was reached. Facilitation, however, was almost completely abolished.

2. Effect of Meprobamate on Recurrent Inhibition

Whereas meprobamate depresses recurrent facilitation, there is usually no decrease in the magnitude of recurrent inhibitory action. The results of meprobamate injection on such inhibitory actions are shown in Table II. In seven instances inhibition, percentagewise, was increased by the drug, while in the other two a small decrease was observed. Variation in the depth of inhibition occurred frequently during the course of the experiment. The most striking effect of meprobamate, however, was an increase in the duration

TABLE II
ACTION OF MEPROBAMATE ON RECURRENT INHIBITION

Experiment	Total dose	Reflex size		Inhibition in absolute units		Inhibition		Inhibition duration	
		Control	At end of experiment	Control	At end	Control	At end	Control	At end
						<i>per cent</i>	<i>per cent</i>	<i>msec.</i>	<i>msec.</i>
1	210	55	26	16	16	29	61	55	>70
2	260	62	37	18	9	29	27	55	>80
3	290	56	39	14	12	25	30	55	>95
4	400	58	26	10	8	18	30	40	>95
5	230	60-65	56-59	37	30	58	49	60	>90
7	240	51-54	14.5	30	10	57	66	50	>80
10	310	47	26	8	7	19	23	40	55-60
12a	280	47	8	3	1	9	13	45	70
12b	280	47	8	9	3	22	36	45	>90

In Experiments 1, 2, 3, and 4 the test reflex was in the nerve to plantaris and inhibition was evoked by antidromic stimulation of the nerves to triceps surae; in 5 triceps surae conditioned flexor longus digitorum; in 7 flexor longus digitorum plus triceps surae conditioned semimembranosus; in 10, extensor brevis conditioned tibialis anterior; in 12a and 12b the deep peroneal nerve and triceps surae respectively conditioned biceps posterior-semi-tendinosus.

of inhibition. Fig. 2 illustrates an experiment in which monosynaptic reflexes in the nerve to semimembranosus were inhibited by antidromic stimulation of the nerves to triceps surae. There is an increase in the depth of inhibition at the time of its maximum but a much greater change is to be seen at longer conditioning-test intervals. Before administration of meprobamate the test reflexes returned to control level at a stimulus interval of about 45 msec. After 200 mg./kg. had been injected, the reflexes were still inhibited to 80 per cent of their unconditioned size at an interval of 80 msec. Increase in duration is usually, but not always, accompanied by an increase in the peak depth of inhibition.

The effect of meprobamate on recurrent inhibition has been studied in experiments in which nerves to flexor and extensor muscles were used for

both conditioning and testing, and an increase in duration of inhibition was observed in all instances.

3. *Action of Meprobamate on Mixed Inhibitory and Facilitatory Effects*

The effect of meprobamate has been tested on mixed conditioning actions consisting of an initial inhibitory phase followed by facilitation. This has been done when a strong inhibitory action was evident before drug administration, as well as in other situations in which only traces of inhibition could be seen. If a mixed effect is present, meprobamate injection leads to a gradual increase in the depth of inhibition. The drug also lengthens the interval between the onset of inhibition and its reversal to facilitation. Simultaneously, the facili-

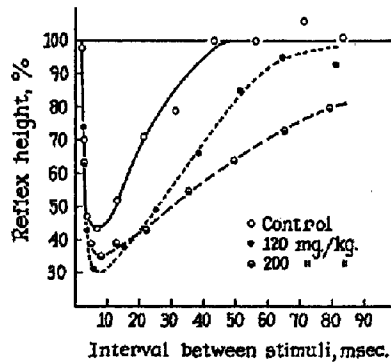


FIGURE 2. Effect of meprobamate on recurrent inhibition. The monosynaptic reflex in the nerve to semimembranosus was inhibited by antidromic stimulation of the nerves to triceps surae. At 120 mg./kg. the test reflex was 72 per cent of control; at 200 mg./kg., 40 per cent.

tation which follows the inhibition is reduced. This result is illustrated in Fig. 3, which shows an instance in which the inhibitory phase was initially very weak. In this experiment deep peroneal reflexes were conditioned by antidromic stimulation of the nerves to triceps surae, flexor longus digitorum, and tibialis posterior. The inhibition became more pronounced with relatively small doses, and by the end of the experiment a fairly typical inhibitory curve was revealed. As usual, the facilitation was practically abolished.

4. *Effects of Nembutal and Meprobamate Compared*

Because it was of interest to compare the effects of meprobamate with those of a commonly used depressant, the effects of intravenously injected nembutal on facilitation, inhibition, and mixed effects, were studied in a few experiments. This drug, in doses of the order of 5 to 10 mg./kg., exerted the same effects on recurrent conditioning as did meprobamate. However, the effects on inhibition and facilitation were accompanied by a much more powerful depression of monosynaptic reflexes than was brought about by the doses of meprobamate that had the same action on conditioning.

DISCUSSION

These experiments demonstrate that recurrent inhibition and recurrent facilitation are differently affected by the action of meprobamate. While variations in the depth of inhibition have been seen in conjunction with meprobamate injection, recurrent inhibition is essentially resistant to meprobamate, as are the direct and disynaptic inhibitory paths (Wilson, 1958*b*). At the end of most experiments the degree of inhibition had changed little and those changes seen were usually increases. In contrast, recurrent facilitation is consistently reduced by the drug. Thus, while recurrent inhibition and facilitation show some pharmacological similarities, both being blocked

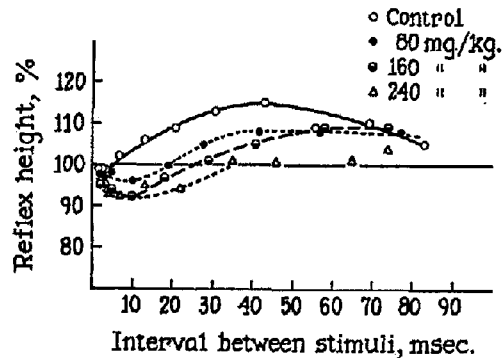


FIGURE 3. Effect of meprobamate on a mixed inhibitory and facilitatory effect. Same experiment as in Fig. 2. The monosynaptic reflex in the deep peroneal nerve was conditioned by antidromic stimulation of the nerves to triceps surae and to flexor longus digitorum plus tibialis posterior. At 80 mg./kg. the test reflex was 86 per cent of control; at 120 mg./kg., 78 per cent; at 160 mg./kg., 73 per cent; at 240 mg./kg., 63 per cent.

partially by dihydro-beta-erythroidine and lengthened by eserine (Brooks and Wilson, 1958; Wilson, 1959), a difference in the pharmacological properties of the two pathways is revealed by the action of meprobamate.

Because of this difference between the effects of meprobamate on recurrent facilitation and inhibition, it has been used as a tool to analyze conditioning effects which are mixtures of the two. Even in situations in which a long lasting facilitation is preceded by a very small and short inhibitory effect, injection of meprobamate uncovers a longer and deeper inhibitory action. The drug may reveal this inhibition either by removing facilitation, or by weakening the latter sufficiently so that it is overcome by the inhibition, itself not depressed by meprobamate. The original conditioning curve may represent the approximate algebraic sum of the separate conditioning actions.

Meprobamate consistently increases the duration of recurrent inhibition. Possibly this is due, at least in part, to the removal of a facilitatory component, which, although usually not strong enough to change the conditioning to facilitation, nevertheless reduces the inhibition and shortens its duration. The difference between the course of inhibition prior to and following a series of injections can be obtained by subtracting the initial from the final

conditioning curve. This has been done for a number of experiments, and Fig. 4 shows the result obtained in the experiment illustrated in Fig. 2. The curve obtained by subtraction strongly resembles a typical recurrent facilitation curve, which suggests that the change in form of inhibitory curves following injection of meprobamate may indeed be partly due to removal of a masked facilitatory action, and hence that the ordinary recurrent inhibitory curve is the final result of a summing of inhibition and facilitation.

If the lengthening of recurrent inhibition is due to the removal of a facilitatory component, then, since lengthening was observed in all cases, it appears that recurrent inhibition is always accompanied by recurrent facilitation. The converse, however, is not true. We have often obtained pure facilitation (*i.e.*, facilitation which disappears following meprobamate or nembutal injection but which does not reverse to inhibition). Possibly the

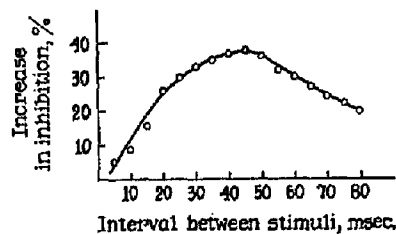


FIGURE 4. Curve obtained from data in experiment of Fig. 2, by subtracting pre-drug inhibitory curve from the inhibitory curve at 200 mg./kg. Further discussion in text.

field of facilitatory influence throughout the motor nuclei is wider than is that of inhibitory influence. This is consistent with the findings that inhibition is prominent between nuclei located close together in the spinal cord (Renshaw, 1941; Eccles, Fatt, and Koketsu, 1954; Brooks and Wilson, 1958), while facilitation is evident when the nuclei involved are not in the same part of the ventral horn (Renshaw, 1941).

The pharmacological data, therefore, suggest that two separate phenomena, recurrent facilitation and recurrent inhibition, are present in various combinations in interactions between different motor nuclei, although both effects need not always be present. Such combinations can account for the various types of recurrent conditioning that have been described (Renshaw, 1941; Lloyd, 1951; Wilson, 1959). In all cases the final shape of the conditioning curve would be determined by the relative latency, magnitude, and duration of the two effects. As recurrent inhibition has a shorter latency and an earlier peak than does recurrent facilitation (Wilson, 1959), it is to be expected when both are present that the former will predominate at short conditioning-test intervals.

A combination of inhibition and facilitation could also account for the results of Granit, Pascoe, and Steg (1957). These authors showed that if the

discharge of a single tonic motoneuron is inhibited by an antidromic shock, the first responses following the period of inhibition may be double or even triple discharges. Similarly, they noted that a reflex discharge evoked by a brief afferent tetanus may be split into two parts by an antidromic shock, the first part of the discharge being inhibited, the latter part accelerated or lengthened. The enhancing action producing doubling or lengthening was observed only in excitable preparations and was never seen without previous inhibition; its threshold was the same as that of inhibition; and, Granit, Pascoe, and Steg (1957) have suggested that it is a rebound from the previous inhibition. While the presence of rebound cannot be ruled out, it is likely that the results of Granit, Pascoe, and Steg (1957) are due to a combination of recurrent inhibition and facilitation. The finding that doubling of motoneuron discharge was never seen without previous inhibition can be explained by the fact that pure facilitation is not easily obtained. It has been seen only between certain nuclei, and not in every experiment (unpublished observations; Wilson, 1959). The close similarity between the thresholds of inhibition and of motoneuron spike doubling is consistent with the finding that the thresholds of recurrent inhibition and recurrent facilitation are, to all intents and purposes, the same (Wilson, 1959).

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