Monosynaptic Transmission in the Cat's Spinal Cord

A Quantitative Measure of Anesthetic Dose

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Amplitude of the monosynaptic reflex potential elicited in a lumbosacral ventral root by supramaximal electrical stimulation of the corresponding dorsal root was measured in seven spinal cats. Infusion of pentobarbital at a steady rate of 1 mg./kg./minute caused a progressive decline in amplitude of the monosynaptic spike which, expressed as a percentage of the control value, was inversely proportional to the logarithm of the cumulative dose of pentobarbital. The amplitude of the monosynaptic reflex evidently assays quantitatively levels of anesthetic depression in the spinal cord.

Assuming that synaptic transmission in other portions of the CNS is not greatly different from that in a spinal cord model, measuring the amplitude of a spinal reflex may provide an objective method to study "depth" of barbiturate anesthesia.

THE NEED FOR QUANTIFYING the depressant action of anesthetics on the central nervous system (CNS) becomes increasingly greater as more refined methods are used to study other systems. For some time it appeared that the electroencephalogram (EEG) would provide an accurate and uniform expression of "anesthetic depth," especially with the development of computer techniques to analyze waveform and frequency.[1] Unfortunately, the EEG patterns produced by the newer halogenated anesthetics are unlike the classical patterns described for ether and cyclopropane.[2]

Eger and his associates [3] have developed a technique for measuring the minimum alveolar concentration of anesthetic (MAC) required to reach a specific endpoint. As this measurement corresponds to light surgical anesthesia, it cannot provide information about depression of the CNS at deeper levels of anesthesia. Although a growing number of investigators are studying anesthetics given in concentrations which are multiples of the MAC value, no evidence exists that, for instance, doubling the anesthetic concentration results necessarily in twice the degree of CNS depression.

In previous studies [4] we observed a strong correlation between depression of the amplitude of a spinal monosynaptic reflex and the clinical signs of increasing depth of anesthesia. In other clinical studies, the amplitude of the monosynaptic spinal reflex was identical at MAC of halothane and at MAC of halothane with nitrous oxide.[5] These observations suggested that the monosynaptic spinal reflex might provide an objective measure of the CNS depressant action of anesthetics at MAC, as well as at deeper levels of anesthesia.

Before proceeding with the study of inhalation anesthetics, it became necessary to determine the degree of correlation between drug dose and depression of central synaptic transmission. For this reason we measured the monosynaptic spinal reflex during the intravenous administration of pentobarbital (Nembutal). Pentobarbital was chosen because it is widely used by neurophysiologists as a standard for comparing other anesthetics. We shall show here that the greater the dosage of pentobarbital, the lower the amplitude of the monosynaptic reflex.

Methods

Seven unmedicated adult cats weighing 3.1–4.2 kg. were anesthetized with halothane given by mask. The trachea was intubated

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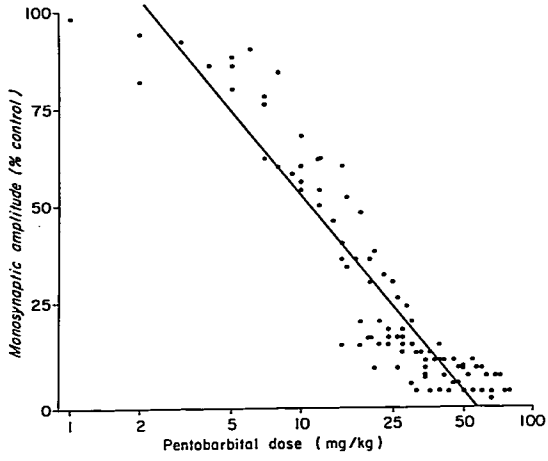

FIG. 1. Amplitude of the monosynaptic spike (percentage control value) plotted against cumulative dosage of pentobarbital (mg./kg.) on a semilogarithmic scale. The computed regression line is shown also.

following topical anesthesia of the larynx. After ligation of the carotid arteries, the spinal cord was transected at the upper cervical level. The lungs were then ventilated mechanically with oxygen at a constant rate and tidal volume (arterial P_{CO_2} between 30 and 40 mm. Hg). Blood pressure in the femoral artery and the electrocardiogram were recorded continuously. Fluid losses were replaced with 5 per cent glucose in lactated Ringer's solution via a catheter in the saphenous vein. Esophageal temperature was maintained between 36.8 and 38.5° C. with a heating blanket.

The lumbosacral segments of the spinal cord were exposed through a lower lumbar laminectomy; the vertebral column was then immobilized. Ipsilateral dorsal and ventral roots of the L6, L7 or S1 segments were cut distally and lifted onto paired platinum electrodes. The dorsal root was stimulated with 0.2–0.3 msec. rectangular pulses at supramaximal (1.5 times maximal) intensity. The resultant ventral root reflex potential was amplified, displayed on an oscilloscope, and photographed on 35 mm. film. The spinal cord and roots were bathed in a pool of mineral oil at 38° C. saturated with 5 per cent CO_2 in oxygen by bubbling.

Following control records of the ventral root reflex potential, pentobarbital was given intravenously at a rate of 1 mg./kg./minute and a volume of 0.2–0.4 ml./minute by means of an infusion pump. Reflex potentials were recorded every one or two minutes until the response was extinguished.

The amplitude and latency of the monosynaptic reflex spike were measured on the projection-enlarged image of the filmed record. Data on amplitude and latency of the monosynaptic spike and cumulative dose of pentobarbital were entered on punch cards. Spike amplitudes and latencies were computed as percentages of their respective control responses; also computed—using Massey's XTAB Program B-6—were the regressions for these values on the cumulative dose of pentobarbital, and their corresponding semilogarithmic and logarithmic functions. Area under the monosynaptic spike—representative of electrical output over the monosynaptic pathways—was computed by rectangular integration of successive segments, each 250 μsec. in length. The systolic and diastolic blood pressures were measured on the paper tracing for each successive 5 mg./kg. dose of pentobarbital, converted

to percentages of control pressure, and plotted against dose of pentobarbital.

Results

The best-fitting least-squares line (fig. 1) was obtained when percentage of the monosynaptic response was plotted against logarithm of the cumulative dose of pentobarbital ($r = -0.930$; $P < 0.001$). Hence the amplitude of the monosynaptic spike is inversely proportional to the logarithm of the total dose of pentobarbital. On the average, the monosynaptic reflex fell to 50 per cent of control after 10 mg., to 37 per cent after 17 mg., to 25 per cent after 25 mg., to 10 per cent after 40 mg., and became flat after 58 mg. of pentobarbital had been given at a rate of 1 mg./kg./minute.*

Coincident with increasing dosage of pentobarbital and falling amplitude of the reflex, latency from stimulus artifact to onset of the reflex response lengthened. A positive linear correlation was found between logarithm of percentage of control latency and cumulative dose of pentobarbital ($r = 0.526$; $P < 0.01$; see fig. 2). Evidently, pentobarbital lengthens synaptic delay in proportion to total dose given.

During the administration of pentobarbital, arterial blood pressure declined slowly but progressively. Nevertheless, when the reflex was extinguished by pentobarbital, systolic blood pressure was usually 75 mm. Hg or greater (in one case it fell to 65 mm. Hg); and not less than 80 per cent of the control pressure (fig. 3). Moreover, microscopic inspection of small vessels accompanying the spinal roots showed vigorous propulsion of blood when the systolic pressure was greater than 60 mm. Hg.

Discussion

We have shown that pentobarbital produces a dose-related depression of the monosynaptic reflex. In other words, pentobarbital inhibits synaptic transmission in a predictable manner. Though pentobarbital is far from an ideal clinical anesthetic, its wide use in neurophysiologic experiments relating to anesthetic action suggested this study. Our observation that the monosynaptic reflex is inversely proportional to the logarithm of the dose of a barbiturate anesthetic lends support to similar studies with inhalation anesthetics.

Because pentobarbital, given at a steady rate of infusion, produced minimal hypotension in spinal cats, we believe that perfusion of the spinal cord remained adequate during these experiments. It appears unlikely that the modest fall in blood pressure contributed to the progressive decline in amplitude of the reflex.

Prolongation of the latency to onset of the ventral root response suggests that pentobarbital also slows the transsynaptic passage of

* Whereas amplitude of the monosynaptic response is easier to measure than area under the monosynaptic spike, the latter reflects more fully the total transsynaptic output over the monosynaptic pathway. A strong correlation was found also between the logarithm of pentobarbital dosage and percentage of control value of area under the monosynaptic response ($r = -0.598$; $P < 0.01$). Hence, area under the monosynaptic reflex response may be used instead of amplitude of the monosynaptic spike to express the depression of synaptic transmission caused by pentobarbital.

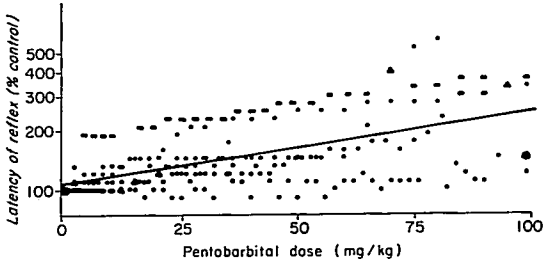

FIG. 2. Latency to onset of the monosynaptic spike (percentage control value) plotted against cumulative dosage of pentobarbital (mg./kg.) on a semilogarithmic scale. The computed regression line is shown also.

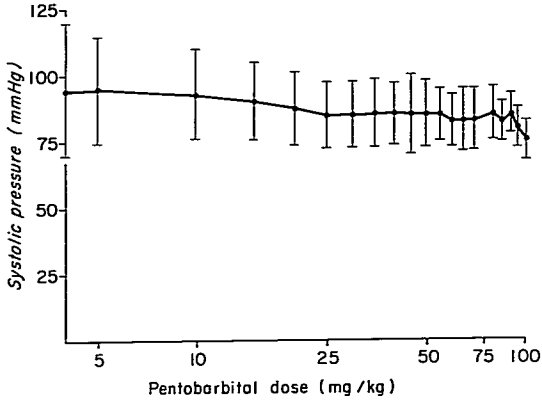

FIG. 3. Mean systolic pressure (mm. Hg ± S. D.) plotted against cumulative dosage of pentobarbital (mg./kg.) on a semilogarithmic scale.

impulses. This action conceivably could be due to drug effect on presynaptic afferent terminals, for reducing afferent input to the motoneuron slows the rise of the excitatory postsynaptic potential.[6] Alternately, transmission could be slowed by drug action on the postsynaptic membrane, as shown by Somjen and Gill [7] for pentobarbital and ether.

We have confirmed the observations of Løyning and his coworkers,[8] who found that the larger the dose of thiamylal (Surital) given intravenously, the lower the amplitude of the monosynaptic spike. For instance, 10 mg./kg. of thiamylal given in one minute reduced the reflex on the average to 26 per cent of control amplitude. This, and their other values, were lower than those found here, possibly owing to faster administration. Other evidence that the monosynaptic response provides specific information about the synaptic depressant action of anesthetics derives from the work by Esplin.[9] This author showed that certain anesthetics, among them pentobarbital, have a selectively greater action on the monosynaptic response than other classes of centrally acting depressant drugs, *e.g.*, mephenesin and trimethadione.

The mechanisms by which pentobarbital and other anesthetics depress synaptic transmission are complex and still largely unknown. By using the spinal animal we excluded descending brainstem and cortical influences acting on the segmental reflex. Even so, anesthetics probably act on several components of the spinal synaptic linkage.[10, 11] Thus, it is by no means certain that the monosynaptic reflex mirrors the depressant action of anesthetics at sites in the CNS other than the spinal cord. Nevertheless, the monosynaptic reflex, since it represents the common end product of drug action on synaptic transmission, may offer some promise of becoming a useful quantitative measure of the central depressant action of anesthetics. Particularly promising is the possibility that a clinical means of quantifying the central action of anesthetics may be found also, as the monosynaptic reflex can be elicited indirectly in man.

Summary and Conclusions

A continuous intravenous infusion of pentobarbital was given at a rate of 1 mg./kg./minute to seven spinal cats. Supramaximal electrical stimulation of a lumbosacral dorsal root elicited a compound reflex potential in the corresponding ventral root. The amplitude of the monosynaptic spike was inversely proportional to the logarithm of the cumulative dose of pentobarbital ($P < 0.001$) and

not related to the modest decline in arterial blood pressure.

This correspondence suggests that measurement of the amplitude of the monosynaptic spinal reflex response is a practical method of quantifying the central depressant action of barbiturates.

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Drugs

PHEOCHROMOCYTOMA Nineteen of 26 patients with pheochromocytoma had positive responses to pharmacologic testing with tyramine, whereas only three of 88 patients with other forms of hypertension had false-positive responses. A positive response was defined as a systolic pressure increase of 22 mm. Hg or greater after intravenous injection of as much as 1,000 μg. tyramine base. Except for palpitation, there were no adverse effects from the tyramine tests. More false-negative responses occurred in patients with the familial variety of pheochromocytoma associated with medullary carcinoma of the thyroid gland than in patients receiving hydrochlorothiazide or phenoxybenzamine. The inhibitory effects of these drugs lasted four days and two weeks, respectively, after they were discontinued. The tyramine test appears to offer specificity as a pharmacologic test for pheochromocytoma comparable to other provocative tests, with the advantages of safety and absence of morbidity. However, all such provocative tests should be used only as screening procedures; they are not substitutes for direct chemical evidence of elevated catecholamine production and release. *(Engelman, K., and others: Further Evaluation of the Tyramine Test for Pheochromocytoma, New Engl. J. Med. 278: 705 (March) 1968.)*