The reasons for the difference in the action of individual drugs belonging to the analgesic group and the benzodiazepine derivatives are not absolutely clear and are difficult to explain at the present time. The possibility cannot be ruled out that the differences are connected with their influence on different mediator mechanisms of the antinociceptive effect [12].

LITERATURE CITED

1. A. V. Val'dman, The Neuropharmacology of Narcotic Analgesics [in Russian], Moscow (1972).
2. A. V. Val'dman and Yu. D. Ignatov, The Central Mechanisms of Pain [in Russian], Leningrad (1976).
3. Yu. N. Vasil'ev, "Effect of analgesics and narcotics on presynaptic inhibition of afferents of different modalities at the segmental level and at the level of the nucleus of the trigeminal nerve," Author's Abstract of Candidate's Dissertation, Leningrad (1975).
4. A. V. Dmitriev, in: The Psychopharmacology of Emotional Stress and of Zoosocial Interaction [in Russian], Leningrad (1975), pp. 29-35.
5. Yu. D. Ignatov and A. V. Dmitriev, Byull. Éksp. Biol. Med., No. 10, 1158 (1976).
6. L. E. Polyakov (Editor), Statistical Methods of Investigation in Medicine and Health Care [in Russian], Leningrad (1971).
7. H. Akil and D. J. Mayer, Brain Res., 44, 692 (1972).
8. C. R. Chapman and B. W. Feather, Psychosom. Med., 35, 330 (1973).
9. J. C. Liebeskind, G. Guilbaud, J. M. Besson, et al., Brain Res., 50, 441 (1973).
10. R. Melzack and D. R. Melinkoff, Exp. Neurol., 43, 369 (1974).
11. R. Samanin and L. Valselli, Eur. J. Pharmacol., 16, 298 (1971).
12. R. Samanin, D. Cherri, C. Mauron, et al., Psychopharmacologia (Berlin), 33, 365 (1973).
13. R. S. Snider and W. T. Niemer, Stereotaxic Atlas of the Cat Brain, Chicago (1961).

EFFECT OF INTRAVENOUS DIAZEPAM ON CORTICAL UNIT ACTIVITY IN RABBITS

S. N. Kozhechkin

UDC 615.214.22.015.4:612.822.3

Changes in single unit activity were studied by a microelectrode technique in the sensomotor cortex of rabbits at different times after a single intravenous injection of diazepam (1-5 mg/kg). A few seconds after the injection of diazepam marked depression of spontaneous activity and of activity evoked by sciatic nerve stimulation was observed, together with an increase in the duration of the inhibitory pause in responses of the neurons to afferent stimulation and to direct stimulation of the cortical surface. These changes were considerably reduced 15-60 min after injection of diazepam. The results were compared with those of other workers who studied the clinical and pharmacokinetic effects of the benzodiazepines. It is concluded that the depressant effect of diazepam on cortical activity is connected with its sedative, amnesic, and anticonvulsant effect, and also that GABA plays an important role in the mechanism of these effects.

KEY WORDS: diazepam; sensomotor cortex; dynamics of depression of unit activity.

In a series of articles devoted to the benzodiazepines the effect of these drugs on electrical activity of nerve cells in different parts of the CNS is described [2, 6, 7, 11, 14]. The data given are not only episodic, but also sometimes contradictory in character. As yet no systematic study has been made of changes in the activity of brain neurons in the course of time after injection of benzodiazepines.

Laboratory of Pharmacology of the Nervous System, Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Zakusov.)

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 85, No. 1, pp. 41-45, January, 1978. Original article submitted June 10, 1977.

0007-4888/78/8501-0046$07.50

©1978 Plenum Publishing Corporation

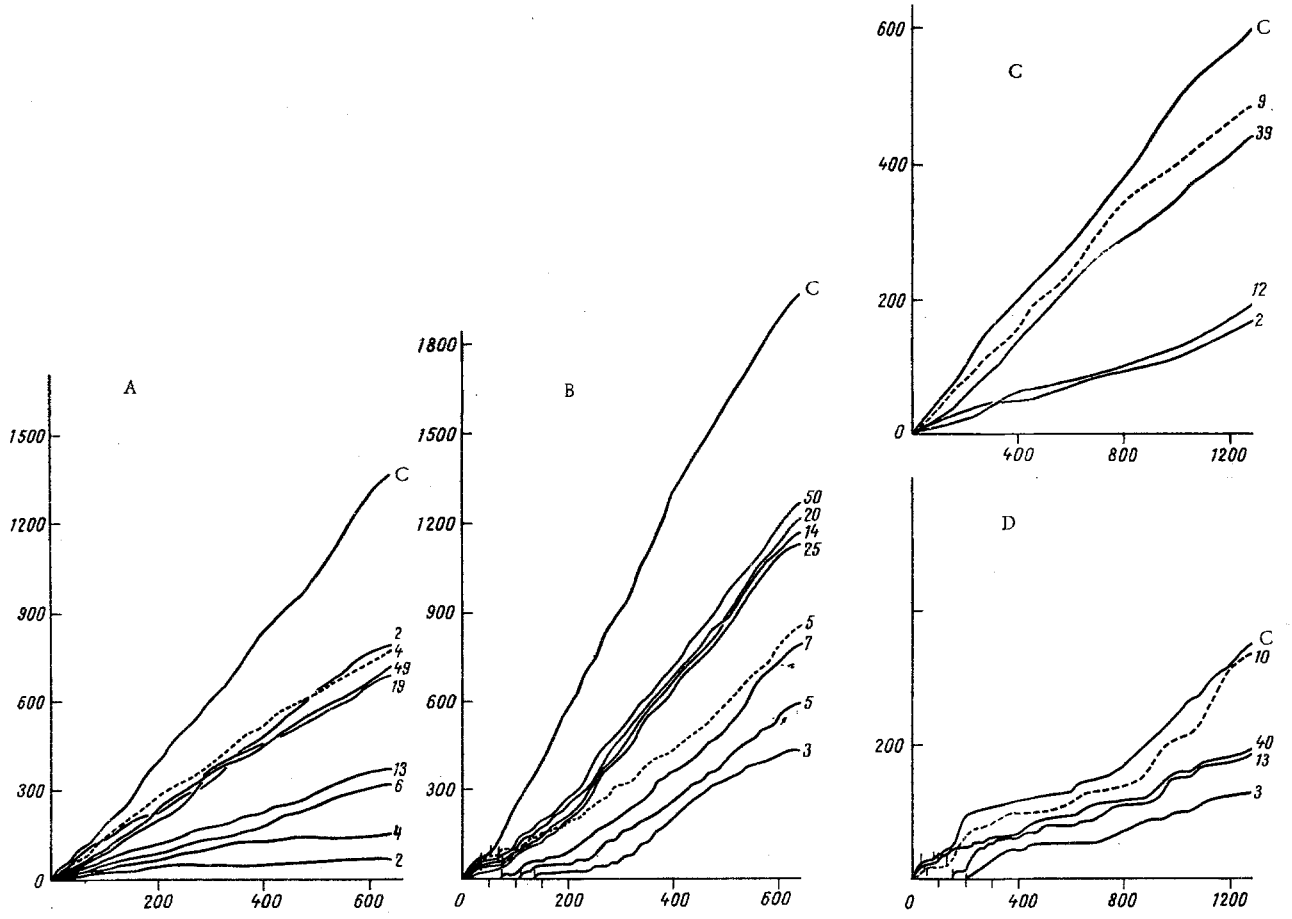

Fig. 1. Changes in different types of electrical activity of two neurons of rabbit sensomotor cortex with time after intravenous injection of diazepam, 1 mg/kg. A, B) one neuron; C, D) another neuron. A, C) Spontaneous activity; B) responses of neuron to electrical stimulation of sciatic nerve (1.50 V). D) Response of neuron to electrical stimulation of cortical surface (3.15 V). Abscissa, time (in msec); ordinate, number of action potentials. C) Control of unit activity before injection of diazepam. Numbers by curves show time after injection of diazepam (in min). Vertical lines on curves show duration of inhibitory pause. Remainder of explanation in text.

The object of the present investigation was to study the temporal pattern of changes in sensomotor cortical unit activity after a single intravenous injection of diazepam. The need for such an investigation has arisen primarily because of the marked effect of benzodiazepines on cortical inhibition recently demonstrated in the writer's laboratory [1, 2, 16]. Moreover, the world literature now contains a wealth of data on the pharmacokinetics of the benzodiazepines and on the temporal course of their clinical effects. It seemed important to compare these data with the results of a dynamic study of changes in cortical unit activity after parenteral injection of benzodiazepines.

EXPERIMENTAL METHOD

Experiments were carried out on 13 adult rabbits weighing 3-4 kg. Sensomotor cortical unit activity was recorded extracellularly by glass microelectrodes filled with 3 M NaCl solution at the focus of maximal activity, identified by stimulation of the sciatic nerve with square pulses (0.1 msec, 0.2-10 V). Besides responses of single neurons to afferent stimulation, their responses to electrical stimulation of the cortical surface (0.1 msec, 5-10 V) through bipolar nichrome electrodes (diameter 0.1 mm, interelectrode distance 0.2 mm) also were studied. The spontaneous unit activity was recorded first (60 sec) followed by 30 responses of the neuron to stimulation of the sciatic nerve or cortical surface (60 sec, 0.5 Hz); this was followed by intravenous injection of diazepam (Seduxen, Gedeon Richter, Hungary) in a dose of 0.5-5.0 mg/kg at the rate of 1-2 mg/min). At various times after injection of diazepam, unit activity was again recorded by the scheme described above. To analyze the data, standard voltage quanta were formed from the neuronal discharges. By summation of each successive quantum with its predecessor, unit activity was represented as a function of voltage with time. Synchronous summation of 30 cuts of spontaneous and evoked activity (640 or 1280 msec) was carried out by

means of the "Neiron-1" analyzer (BIOCODE-1 program). The data thus averaged were printed out by automatic writer. As a result, the unit activity was represented by a line whose angle of inclination to the abscissa was directly proportional to the discharge frequency. Other details of the method were described previously [1].

EXPERIMENTAL RESULTS

Intravenous injection of diazepam in a dose of 1 mg/kg or more reduced the spontaneous firing rate of all neurons studied (21 cells). A marked decrease in the slope of the curves of spontaneous activity 2-5 min after the injection of diazepam will be seen in Fig. 1A, B and Fig. 2A. Later the spontaneous activity gradually recovered. The slope of the curves increased with an increase in time after the injection of diazepam. The comparatively rapid recovery of spontaneous activity usually continued for the first 20-40 min after injection (Fig. 1A, B). At the end of the recovery period spontaneous activity was established at a definite level, which was always a little below the initial level (before injection of diazepam). The activity remained at this level for as long as the action potential of the same neuron could be recorded (up to 90 min).

As Fig. 1B shows, sciatic nerve stimulation activated the neuron (the slope of the C curve is greater than the slope of the C curve in Fig. 1A). Against the background of this activation an inhibitory pause can be seen – a region of the curve with a small angle of slope lasting 25 msec. Marked inhibition of the unit response combined with an increase in the duration of the inhibitory pause to 130 msec were observed 3 min after the injection of diazepam. During the subsequent period of observation the initial characteristics of the evoked responses were restored. After 14 min recovery ceased and the responses became stabilized at a certain level with no tendency to change thereafter. This level was lower than the control level of evoked activity. The inhibitory pause differed only a little from that before injection of diazepam.

The effect of diazepam on the response of a neuron to cortical electrical stimulation is shown in Fig. 1D. Before injection of diazepam the response was characterized by a reduction in the discharge frequency. The response included an inhibitory pause lasting 50 msec. Inhibition of unit activity and an increase in the duration of the inhibitory pause to 200 msec were observed 3 min after injection of diazepam (1 mg/kg). By 13-40 min the response had recovered to some degree and become stabilized at a definite level which differed from the initial level in its lower discharge frequency. The duration of the inhibitory pause 40 min after injection of diazepam was no longer significantly different from the initial value.

After injection of diazepam in doses exceeding 1 mg/kg (2-5 mg/kg) the characteristics of the change in spontaneous and evoked cortical unit activity were similar to those described above. However, restoration of both types of activity after the initial depression took place more slowly than when small doses of diazepam were given. As Fig. 2 shows, by 23-27 min after injection of diazepam (3 mg/kg) inhibition of spontaneous and of both forms of evoked activity still remained strong. Recovery of unit activity usually took place between 40 and 60 min after injection of large doses of diazepam.

It will be clear from the experimental results that diazepam, given by intravenous injection in a dose of 1 mg/kg or more, has a strong inhibitory action on sensomotor cortical unit activity. The spontaneous discharge frequency of the neurons is reduced and their responses to afferent stimulation contain fewer discharges. In particular, it must be pointed out that the depressant action developed very rapidly in all the experiments: within a few seconds after injection of diazepam, and often actually during the injection. Other workers also found an inhibitory action of diazepam on unit activity in the mesencephalic reticular formation [11], thalamus [6], amygdala [14], cerebellum [7], and cerebral cortex [2].

After a single intravenous injection of diazepam pharmacological effects such as sedative, muscle-relaxing, amnesic, and anticonvulsant (stopping epileptic fits) in man and animals are known to develop within a few seconds after the beginning of the injection and to last for 20-60 min [3, 5, 8]. The considerable inhibition of unit activity now observed during the first 20-60 min after the injection thus develops parallel with the acute pharmacological effects of diazepam and is evidently to some extent responsible for them.

These observations are in good agreement with the results of pharmacokinetic investigations which showed that diazepam, a few seconds after its intravenous injection, penetrates into the gray matter of the brain [9, 10, 15]. The concentration of diazepam in the blood and brain falls considerably 30-60 min after its injection, whereas the concentration of products of its metabolic demethylation and hydroxylation increase at the same time [9, 10, 13, 15]. Metabolites of diazepam circulate for a long time (several hours) in the blood stream and are eliminated more slowly than diazepam itself [13, 15]. These metabolites and, in particular, N-demethyldiazepam, have pharmacological activity qualitatively similar to that of diazepam and they also produce effects such as sedative, tranquilizing, and anticonvulsant (a reduction in the likelihood of occurrence

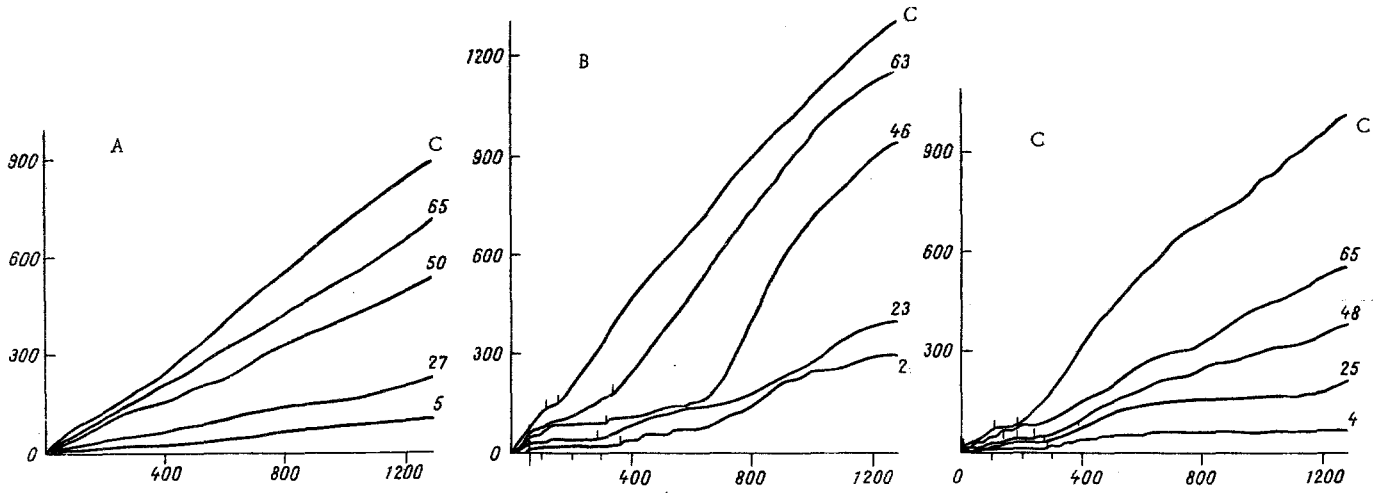

Fig. 2. Effect of diazepam (3 mg/kg) on cortical unit activity. A) Spontaneous activity, B) responses of neuron to electrical stimulation of sciatic nerve (1.75 V), C) responses of neuron to electrical stimulation of cortical surface (2.47 V). Remainder of explanation as in Fig. 1.

of convulsions) which continue for a long time after the injection of diazepam [4, 8, 12]. The residual inhibition of unit activity still observed in the late stages after injection of diazepam in the present experiments corresponds in time with the formation and biological action of diazepam metabolites and also with the manifestation of the long-term clinical effects of the drug mentioned above. The most characteristic feature of the action of diazepam on evoked unit responses was an increase in the duration of the inhibitory pause. It must be specially emphasized that the duration of the inhibitory pause a comparatively short time after the injection (15-60 min) returned almost to its initial value (before injection of diazepam). The writer concluded previously that the increase in the duration of the inhibitory pause after injection of diazepam was due to potentiation of the synaptic action of γ-aminobutyric acid (GABA) [1]. On the basis of the facts stated above it seems reasonable to infer that an important role in the development of the many acute clinical effects following immediately after a single intravenous injection of diazepam is played by potentiation of inhibitory GABA-ergic processes in the neocortex.

LITERATURE CITED

1. S. N. Kozhechkin and R. U. Ostrovskaya, Byull. Éksp. Biol. Med., No. 12, 1448 (1976).
2. R. U. Ostrovskaya and G. M. Molodavkin, Byull. Éksp. Biol. Med., No. 9, 1073 (1976).
3. J. W. Dundee and S. K. Pandit, Br. J. Pharmacol., 44, 140 (1972).
4. S. Garattini, E. Mussini, F. Marcucci, et al., in: The Benzodiazepines, New York (1973), p. 75.
5. H. Gastaut, R. Naquet, R. Poire, et al., Epilepsia (Amsterdam), 6, 169 (1965).
6. R. Guerrero-Figueroa, D. M. Gallant, C. Guerrero-Figueroa, et al., in: The Benzodiazepines, New York (1973), p. 489.
7. W. Haefely, A. Kulcsar, H. Möhler, et al., in: Mechanism of Action of Benzodiazepines, New York (1975), p. 131.
8. I. Hendel, P. Elsass, P. B. Andreasen, et al., Psychopharmacologia, 48, 11 (1976).
9. F. Marcucci, R. Fanelli, E. Mussini, et al., Eur. J. Pharmacol., 9, 253 (1970).
10. P. L. Morselli, G. B. Cassano, G. F. Placidi, et al., in: The Benzodiazepines, New York (1973), p. 129.
11. A. C. Przybyla and S. C. Wang, J. Pharmacol. Exp. Ther., 163, 439 (1968).
12. L. O. Randall and B. Kappell, in: The Benzodiazepines, New York (1973), p. 27.
13. M. A. Schwartz, B. A. Koechlin, E. Postma, et al., J. Pharmacol. Exp. Ther., 149, 423 (1965).
14. M. Umemoto and M. E. Olds, Neuropharmacology, 14, 413 (1975).
15. E. van der Kleijn, Arch. Int. Pharmacodyn., 178, 193 (1969).
16. V. V. Zakusov et al., Arch. Int. Pharmacodyn., 214, 188 (1975).