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Neurophysiological Approach as a Tool to Study Effects of Drugs on the Central Nervous System: Dose–Effect of Ketamine[1]

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The effects of different doses of ketamine on acoustic evoked responses were studied in four brain sites in freely behaving rats. Permanent semi-microelectrodes (60 μm in diameter) were implanted stereotaxically under pentobarbital anesthesia within the inferior colliculus, the primary relay site for acoustic input; within the mesencephalic reticular formation; in the ventromedial hypothalamus; and within the somatosensory cortex. After recovery from surgery (6 to 8 days), the averaged acoustic field potentials following 32 repetitive stimuli were averaged to one trace. Four traces were recorded at 10-min intervals before and after each injection. Ten doses of ketamine were studied (1, 5, 10, 20, 40, 60, 80, 100, 120, and 140 mg/kg ip). The dose which induced surgical anesthesia was found to be 80 mg/kg ip. Differences in the number of cases in which ketamine induced changes in sensory field potentials were observed between structures. The evoked responses in the somatosensory cortex exhibited dose-dependent changes. The lower doses of ketamine below the anesthetic dose (80 mg/kg) induced depression of the responses, whereas higher doses (100 to 140 mg/kg) induced increases in the response amplitudes, shifted the amplitude to the right (long latency), and prolonged the duration of the responses. However, the responses in the sensory relay nuclei were affected by the initial dose of 1 mg/kg and remained about constant following the remaining doses. The reticular formation and ventromedial hypothalamus were affected differently by ketamine. There was demonstrated the possibility that acoustic evoked responses from the somatosensory cortex can be used as an indication of the level of ketamine anesthesia.

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INTRODUCTION

The vast majority of investigations dealing with neurophysiological mechanisms of anesthesia has concentrated on barbiturates. Little work, however, has been done on ketamine hydrochloride (2-orthochlorophenyl, 2-methylaminocyclohexanone HCl [Cl-581]) which was introduced for induction of anesthesia. There seems to be little doubt that ketamine is a powerful analgesic and anesthetic with an unusual spectrum of pharmacological effectiveness. Ketamine is well tolerated by the tissues, has unusual effectiveness and safety, has the characteristics of speed of onset and short induction of anesthesia, and is short-acting (1, 3, 10, 12, 14–16, 18).

Among investigators there is disagreement about the mechanism of ketamine anesthesia (3, 10, 11, 12, 14, 16, 19, 22–24). Ketamine alters the reactivity of the central nervous system to various sensory impulses but does not produce a true sensory blockade (8); it does not block primary sensory input at spinal or brain stem levels. The motor reflexes are intact and frequently hyperactive. Sensory input may reach cortical receiving regions but fail to be perceived in some of the association regions because these are depressed. This interference in proper association of afferent impulses results in "dissociation." It was suggested, therefore, that the state induced by ketamine be called "dissociative anesthesia" (1, 18).

The present study was initiated to study the effects of ketamine simultaneously on four structures, the reticular formation, hypothalamus, and somatosensory cortex, and a sensory relay nucleus, the inferior colliculus. Acoustic input was used as a sensory modality and as a tool to initiate synchronized electrical activity in unanesthetized rats previously implanted with permanent electrodes. No systematic study of ketamine having been conducted, in the present study 10 different doses of ketamine were examined, from very low (1 mg/kg) to very high (140 mg/kg). The "anesthetic dose" was reported as 10 mg/kg intraperitoneally (i.p.) in rats (21). Our studies were conducted in freely behaving preparations, which is essential in studies of the effects of drugs and special effects of anesthetic agents on the central nervous system.

MATERIALS AND METHODS

The methods and procedures performed in the present study were described in detail before (6). In brief, 44 male Sprague–Dawley rats weighing 250 to 350 g were used. The animals were anesthetized with pentobarbital (50 mg/kg ip). Nichrome electrodes (60 μm in diameter) with an uninsulated area consisting of the cut cross section of the tips (area 2.8×10^{-3} mm^2) were implanted stereotaxically (13) in the inferior colliculus, mesencephalic reticular formation, ventromedial hypothalamus, and somatosensory cortex.

Several days (5 to 8) after surgery, the rat was placed in a plastic cage within a Faraday chamber. The electrodes were connected to a Grass P511 preamplifier through a commutator and a counterbalanced arm, which allowed the animal to move freely. Electrical activity was displayed on a storage oscilloscope and connected on-line with a signal-averaging computer (NIC 1070). Thirty-two evoked responses were averaged following acoustic stimuli in the form of clicks and were plotted as one trace with an x–y plotter. Four traces were obtained before and after each injection. Stimulation, recording, histology, and evaluation procedures were similar to those used previously (6). Amplitudes of the individual components of the averaged potentials were measured peak to peak. Responses to the four control stimulus trains were averaged and standard errors (SE) were calculated. Total responsiveness after ketamine administration was evaluated by comparison of component amplitudes averaged over the four post-treatment recordings. Amplitude changes of $\pm$ 2 SE in comparison to control were considered significant.

Animals were tested twice with a 1-week interval between experiments. In each experiment, five random incremental doses of ketamine were given at 1-h intervals in volumes of 1 mg/kg. Four recordings before and following drug administration were taken at 15, 25, 35, and 45 min after each injection. In 20 animals only single doses of the drug were given to identify any differences between single and cumulative injections.

Only the recordings from those electrodes which were shown by microscopic examination to be located in the target nuclei are reported.

RESULTS

Control Predrug Recordings. The averaged acoustic evoked responses following 32 repetitive stimuli differed in latency and amplitude between the four structures examined in the present study (Figs. 1–4). The four control recordings in each central nervous system site demonstrated minor fluctuations with time. The patterns of the evoked responses remained essentially the same for a period of several hours or days (6), and consisted of an initial spike (positive–negative, P_1 and N_1) succeeded by a triphasic wave (P_2, N_2, and P_3). Components P_2, N_2, and P_3 were consistent and were measured individually for statistical evaluation (see Materials and Methods).

Dose–Effects of Ketamine on Field Potentials Recorded from the Inferior Colliculus. At 1 mg/kg ip, ketamine caused significant changes in the response amplitudes in two-thirds of the animals (Fig. 1, Table 1) for the late components (P_2, N_2, and P_3). However, the two earlier components (P_1 and N_1) showed only in a few animals statistically significant modifications even after 140 mg/kg ketamine.

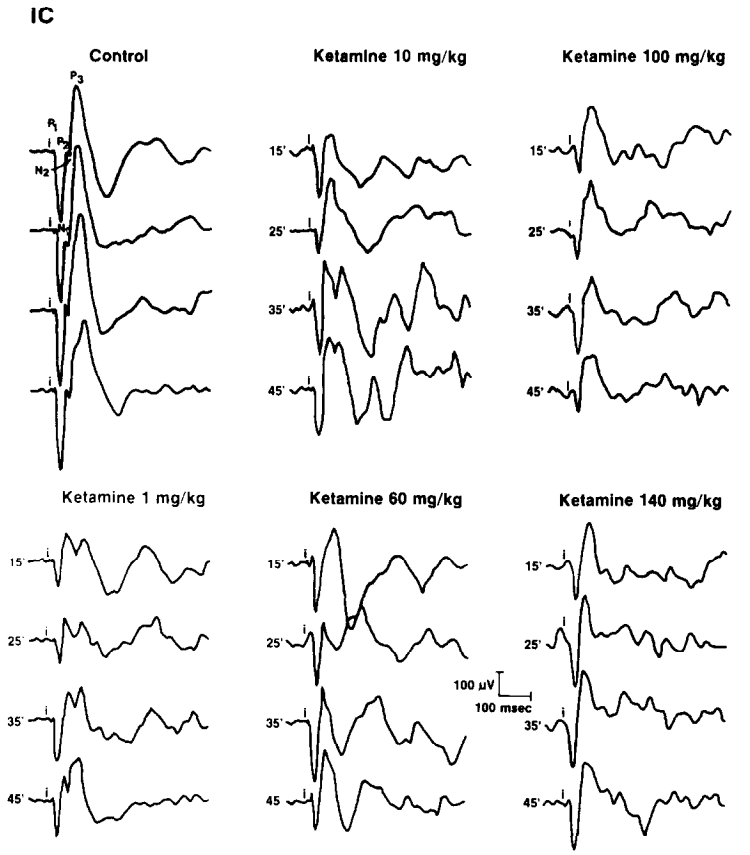

FIG. 1. Representative recording of averaged acoustic evoked responses recorded from the inferior colliculus (IC). Upper-left column (control) presents four predrug recordings. Each trace consists of the averaged responses of 32 repetitive click stimuli, i indicates onset of stimulus, P_1 indicates the initial positive peak followed by N_1, the first negative, and a triphasic wave, positive–negative–positive, P_2, N_2, and P_3. The numbers indicate the recording time after ketamine injection.

During the 45 min of monitoring the electrical activity after any dose of ketamine, no recovery from the drug effects was observed (Fig. 1). With increasing doses, the late components (P_2, N_2, and P_3) in several animals demonstrated slight increases in the response amplitudes compared to the responses obtained during the effects of ketamine in lower doses. In a few cases, the amplitudes were similar to those observed in control recordings but response latencies shifted to the right (Fig. 1). Because Table 1 summarizes the amplitudes without relation to latencies, it shows relatively few significant changes of N_2 and P_3 induced by the largest doses of ketamine. The effect induced by ketamine was mainly depression of components N_2

TABLE 1

Summary of the Total Responsiveness from Four Brain Regions following Incremental Doses of Ketamine[a]

		Ketamine (mg/kg)									
		1	5	10	20	40	60	80	100	120	140
IC[b]	P_2	66	66	38	67	75	64	67	60	64	67
	N_2	66	66	38	78	88	73	67	70	64	50
	P_3	66	66	75	89	88	82	67	60	64	42
RF	P_2	20	25	14	22	13	62	67	67	83	100
	N_2	60	50	21	44	50	69	83	83	83	100
	P_3	80	75	71	78	50	77	83	67	100	100
VMH	P_2	40	40	40	33	25	21	40	67	90	100
	N_2	40	40	50	56	75	78	100	80	100	100
	P_3	100	100	100	89	88	71	60	60	50	50
SC	P_2	20	22	30	36	36	57	67	67	78	100
	N_2	20	33	40	36	45	50	67	75	89	100
	P_3	40	44	50	55	64	71	100	100	100	100

[a] Percentage of animals affected. For the direction of the response (increase or decrease) see text.

[b] IC—inferior celliculus; RF—mesencephalic reticular system; VMH—ventromedial hypothalamus; SC—somatosensory cortex.

and P_3. However, component P_2 was affected similarly with all the doses tested in the present study. At the initial three doses of ketamine (1, 5, and 10 mg/kg), all recordings demonstrated decreases in the response amplitudes, whereas with the higher doses (20 to 140 mg/kg), about half of the responses demonstrated increases in their response amplitudes. No clear dose–response relationships were observed in the average acoustic evoked responses recorded in this brain site (Fig. 1, Table 1).

Dose–Effects of Ketamine on Field Potentials Recorded from the Reticular Formation. Components P_2, N_2, and P_3 of the evoked responses were relatively consistent whereas the early components P_1 anl N_1 showed more variability. Therefore, only components P_2, N_2, and P_3 of the amplitude response were measured and evaluated statistically. Each component was affected by ketamine to different degrees (Fig. 2, Table 1). Component P_2 was the least affected by ketamine in doses to 60 mg/kg (Table 1). Component N_2 was more affected and demonstrated biphasic dose-related effects, and component P_3 showed about equal significant alterations induced by ketamine for most of the doses used (Table 1). The effects induced by ketamine in the lowest dose (1 mg/kg) lasted about 25 min (Fig. 2). At doses of 5 and 10 mg/kg, ketamine induced its effects for 35 min before the acoustic

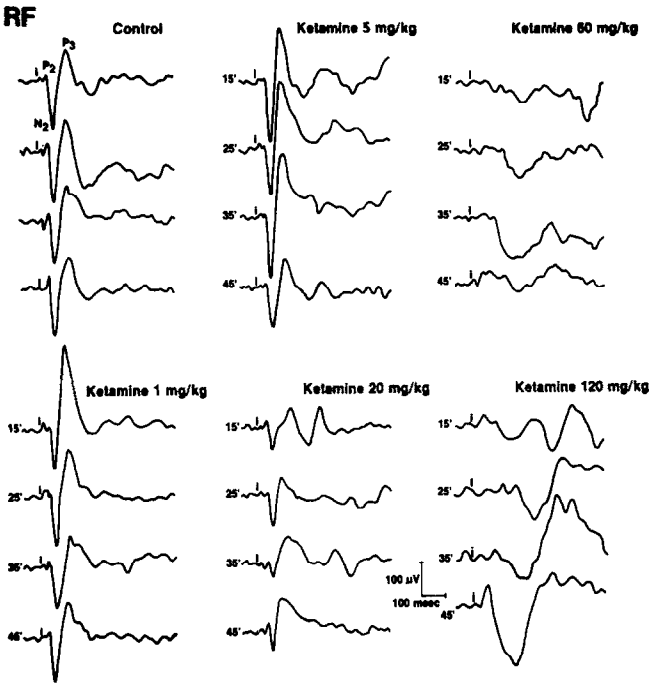

FIG. 2. Presentative recording of averaged acoustic evoked responses recored from the reticular formation (RF).

evoked response recovered from the drug. With doses of 20 mg/kg or greater, no recovery was observed during the 45 min of the recording procedure (Fig. 2). Ketamine at doses of 1 to 80 mg/kg caused mainly increases in the amplitudes of component P_2, and at doses of 100 to 140 mg/kg all response components were depressed. Component N_2 demonstrated a triphasic effect: At doses of 1 to 40 mg/kg, the majority of the response amplitudes was depressed; at doses of 60 to 100 mg/kg the majority of the responses exhibited increases in their amplitudes; and doses of 120 and 140 mg/kg ketamine caused depression of all responses of component N_2. Component P_3 was mainly depressed after ketamine injection, except for doses of 100 and 120 mg/kg, where in several cases this component increased in amplitude and its latency shifted to the right (Fig. 2).

Dose–Effects of Ketamine on Field Potentials Recorded from the Ventromedial Hypothalamus. Each component (P_2, N_2, and P_3) of the acoustically evoked response was affected by ketamine to different degrees (Fig. 3, Table 1). Component P_2 was the least affected by ketamine in doses to 80 mg/kg (Table 1). Component N_2 was more affected and exhibited dose-related effects. Component P_3 with the three smallest doses was altered by ketamine in all animals, but with increasing doses ketamine induced changes

in fewer and fewer animals (Table 1). The effects induced by 1, 5, and 10 mg/kg ketamine persisted during 25 min. No recovery from doses of 20 mg/kg or greater was observed during the 45 min of the recording procedure (Fig. 3). The direction of the significant changes (increase or decrease) was different among the components. Ketamine in doses of 1 to 10 mg/kg in all cases caused increased response amplitudes of component P_2, and at doses of 20 to 80 mg/kg about 50% increases and 50% decreases occurred. From 100 mg/kg and higher, all amplitudes were depressed. However, component N_2 was about equally affected by the different doses of ketamine; 70% of the cases demonstrated attenuation in their responses, and 30% exhibited increases in their response amplitudes. Doses of 1 to 80 mg/kg of ketamine attenuated, whereas doses of 100 to 140 mg/kg increased the amplitudes of component P_3.

Dose–Effects of Ketamine on Field Potentials Recorded from the Somatosensory Cortex. Dose-related effects in the present study were observed in this structure (Table 1). Components P_2 and N_2 responded to about the

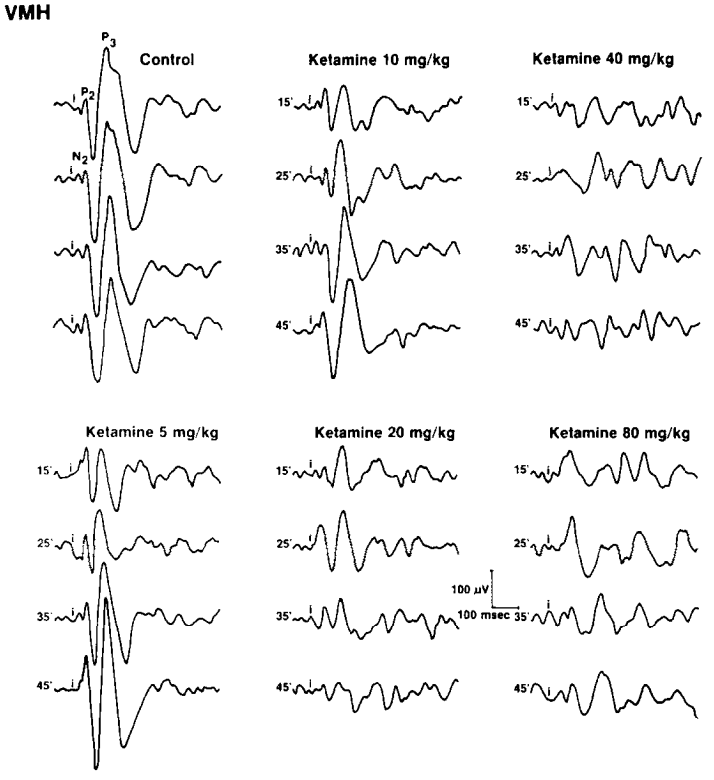

FIG. 3. Representative recording of averaged acoustic evoked responses recorded from the ventromedial hypothalamus (VMH).

same degree, 20% of the cases with the lowest dose (1 mg/kg); the effect was incremental until the dose of 140 mg/kg which affected 100% of the animals (Table 1). Component P_3 demonstrated more sensitivity to ketamine; at the dose of 1 mg/kg, 40% of P_3 responses were affected by ketamine, and 100% of P_3 responses were affected by doses of 80 to 140 mg/kg. Recovery during the 45 min after ketamine injection was observed only with the two smallest dosages. The direction of the changes in amplitudes of response components P_2 and N_2 was similar; the majority of the amplitudes was increased after the first four injections (1 to 20 mg/kg), but depressed after the higher doses. However, component P_3 was depressed in all animals at doses of 1 to 80 mg/kg. After the largest three doses (100 to 140 mg/kg), the majority of the recordings demonstrated increases in their averaged acoustic evoked responses.

In 20 animals only a single dose of ketamine was given. In general, the same pattern of response to a particular dose was observed in these animals compared to those receiving incremental injections of ketamine.

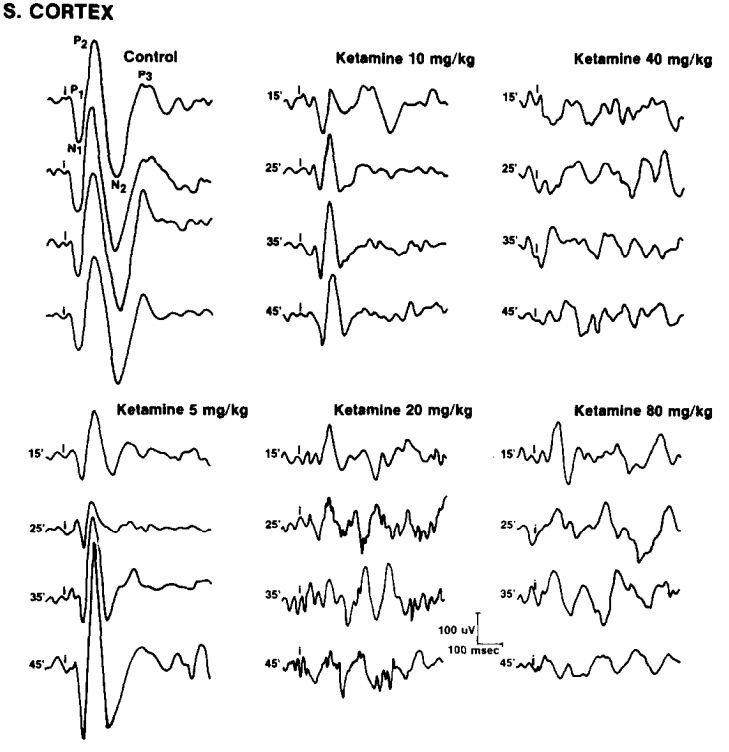

FIG. 4. Representative recording of averaged acoustic evoked responses recorded from the somatosensory cortex (SC).

No animals died during the present experiment. The dose which caused anesthesia was reported to be 10 mg/kg ketamine (21); in the present experiment we therefore used 1 mg/kg as the lowest dose. No anesthesia was observed with the dose of 10 mg/kg and larger doses were given. Because the main clinical use of an anesthetic agent is to perform surgical procedures, in a separate experiment 24 animals were injected with different doses of ketamine and were placed in a stereotaxic instrument. Only with at least 80 mg/kg ketamine was it possible to put the animals in the instrument and to carry out surgical procedures.

DISCUSSION

Ketamine was reported to induce anesthesia of short duration and produce marked analgesia without significant respiratory and cardiovascular depression (2, 3, 14, 18, 21, 22). The passage of time and injection of saline, in other studies using the same technique did not modify sensory field potentials (6, 7). Barbiturates (4–6) and drugs other than ketamine (4, 7) modify the sensory field potentials differently from the effects observed in the present study. Therefore, it is possible to conclude that the changes in the average acoustic evoked responses induced by ketamine in the present are relatively specific for this drug.

The findings of major interest in the present experiments are (i): The most sensitive anatomic region to ketamine is the inferior colliculus, one of the primary acoustic relay nuclei; (ii) in general, the reticular formation, the hypothalamus, and the somatosensory cortex were affected by ketamine to about the same extent; (iii) dose-related responses were observed mainly from 20 to 140 mg/kg in the reticular formation, hypothalamus, and somatosensory cortex; (iv) there were significant differences between the effects of ketamine on the various responses of each component (P_2, N_2, or P_3) within a structure and between structures; and (v) component P_2 in the reticular formation, ventromedial hypothalamus, and somatosensory cortex exhibited biphasic patterns—at lower doses it demonstrated increases in the response amplitudes, and at higher doses of ketamine it was depressed.

Components P_1 and N_1 in the inferior colliculus may monitor the presynaptic events (17, 20); it is generally agreed that these components are related to the arrival of efferent volleys. Ketamine in the majority of cases did not modify those components; thus, it may not modify sensory input at the level of the primary sensory site. This observation is in agreement with those reported by others (1, 2). However, the late components (P_2, N_2, and P_3) were modified markedly, and to a similar degree for all doses tested in this study. It is possible to postulate that the response amplitudes re-

maining after the ketamine injection are due to postsynaptic activity generated in the inferior colliculus whereas the contribution of the higher centers to the late components is attenuated by ketamine via descending connections which influence (control) the activity of this structure.

Each structure responded differently (total responsiveness as well as direction of responses) to the different doses of ketamine and demonstrated markedly different patterns of responses from those obtained following barbiturate (3–6, 15). The mechanism of action of ketamine is different from that of barbiturates and provides an indication that all the reticular formation, ventromedial hypothalamus, and somatosensory cortex are participants in the ketamine effect, with each structure taking a different role. This observation is in agreement with the suggestions of several investigators (9, 12, 16, 24) but is in disagreement with others (2, 3, 15) who reported that ketamine induced patterns of responses in the reticular formation different from those in the hypothalamus or somatosensory cortex. No study has examined systematically the different doses of ketamine, and in the majority of those experiments restrained preparations were used and the definition of anesthesia was different (hot plate, shock) from ours. This can explain most of the differences observed here.

The somatosensory cortex responded to different doses of ketamine with a dose–response pattern; component P_3 was maximally affected at the dose which induced surgical anesthesia. Component N_2 demonstrated triphasic responses, i.e., excitation, depression, and excitation, similar to the observations of Mori *et al.* (16). Component P_2 exhibited depression in subanesthetic doses, while higher doses caused an increase. The results obtained from the somatosensory cortex indicate that the acoustic evoked response can be used as an objective technique to monitor the level of ketamine-induced anesthesia. This observation is of importance because heretofore there has been no routine way to identify the depth of ketamine anesthesia. In previous studies additional administration of barbiturate above the surgical anesthetic dose killed all the animals (6). In the present study however, not one animal was lost even with the highest dose of 140 mg/kg ketamine, which is an indication that ketamine is a very safe agent.

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