The Neural Mechanism of Cyclopropane Anesthesia in the Rabbit

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The effects of cyclopropane on CNS electrical activity were studied in chronic rabbit preparations. Changes in arterial blood pressure and gross behavior were correlated with changes in EEG patterns in the dorsal hippocampus and neocortex and changes in multiple-neuronal-unit activity in the midbrain reticular formation. Cyclopropane induced a biphasic response: initial CNS excitation, followed by depression. Arterial hypertension and bizarre mastication occurred during excitation. While both excitation and the succeeding depression alerting or noxious stimulation did not induce further changes in CNS electrical activity or gross behavior. A metabolic process, possibly involving brain catecholamines, is suggested as the cause of the excitation. Cyclopropane should be classified as a CNS-excitatory anesthetic. General anesthesia or the state of unconsciousness may be associated not only with depression of reticular neurons, as with barbiturates or halothane, but also with excitation. (Key words: Cyclopropane; Reticular multiple-unit activity; Hippocampal theta rhythm; Arterial hypertension.)

At least two kinds of electrical activities can be recorded in the CNS: so-called "spontaneous" or "background" activity, and that which follows peripheral stimulation. Spontaneous activity is that recorded when the external condition of the animal is maintained at basal levels with little or no afferent input. It is more or less tonic in nature. Activity induced by stimulation is time-related and phasic. EEG responses in the reticular formation induced by peripheral stimulation are selectively blocked by general anesthetics, as are cortical EEG arousal responses to high-frequency reticular stimulation. On this basis, depression of reactive capability of the reticular formation has been considered a neural basis of general anesthesia.1-4

Measuring the spontaneous activity of a large population of neuronal elements has enabled this laboratory to demonstrate that anesthetics such as pentobarbital, ether, and halothane depress spontaneous firing of the reticular neurons as well as the EEG response evoked by auditory stimulation,5 8 while anesthetics such as ketamine, phencyclidine, gamma-hydroxybutyrate, and alpha-chloralose induce dysthyrhythmia of neuronal elements in the reticular core.5 8 We have postulated that cyclopropane induces excitation of the brain-stem arousal system, since the rabbit hippocampus during cyclopropane anesthesia showed continuous theta rhythm similar to that seen during consciousness.10, 11

In the present study we attempted to confirm the hypothesis that cyclopropane-induced hippocampal theta activity was the result of excitation of the brain-stem arousal system. Changes in reticular multiple-unit activity (MUA) were correlated with changes in arterial blood pressure, gross behavior, and EEG patterns.

Methods

Fifteen adult albino rabbits of either sex, weighing 2.2 to 3.8 kg, were used. Electrodes had been implanted chronically in the brain of each rabbit for more than ten days prior to study. Surgical procedures for electrode implantation were performed using pentobarbital anesthesia (40 mg/kg im). The structures in
which electrical activity was monitored were: cerebral cortex (A:10, L:2 and A:2, L:2); dorsal hippocampus (P:4, L:5, H:5); midbrain reticular formation (P:3–5, L:3–5, H:3–5). The depth electrodes were placed according to the stereotaxic atlas of Sawyer et al.\textsuperscript{12} (figs. 1 and 2). The cortical electrodes consisted of stainless steel screws 2 mm in diameter. For each depth electrode two epoxide-insulated stainless steel wires 0.2 mm in diameter were twisted together and bared of insulation at the distal end. The recording surfaces were separated by 0.5–1.0 mm.

For each rabbit control measurements were made during wakefulness, slow-wave sleep, and the paradoxical phase of sleep, to permit comparison of drug-induced changes in electrical activities with those observed in various physiologic states. After the control values had been established the cyclopropane experiments were performed.

The rabbit was fixed to a rabbit experimental board with the aid of 2 per cent halothane administered by insufflation. Tracheos-
tomy was done; femoral catheterization of gal-
lamine-immobilized rabbits was also done at
this time. Lidocaine (2 per cent) was painted
with a hair brush on the wound surfaces of the
tracheotomy and femoral catheterization
every two to three hours. The total amount of
lidocaine used in the course of six to ten
hours was estimated to be 4–7 ml. After tra-
cheotomy and administration of local anes-
thesia, halothane insufflation was discontinued.

In six rabbits the relationships between
changes in the EEG patterns, levels of mul-
tiple-neuronal-unit activity in the reticular for-
mation, and arterial blood pressures were
studied. In each of these animals a teflon
catheter (19-gauge) was inserted into the
abdominal aorta through the femoral artery for
measurement of arterial blood pressure and
gas tensions. The femoral nerve was cut
about 1 cm distal to the inguinal ligament.
The rabbit was then immobilized with 10 mg
of gallamine, iv, and an additional 90 mg, sc.
Constant-volume ventilation with room air was
delivered by a mechanical ventilator (Acoma)
so that arterial blood gases and pH remained
constant in the ranges of \( P_{\text{CO}} \) 27.5 to 37.3
mm Hg; \( P_{\text{O}} \) 75 to 105 mm Hg; pH 7.34 to
7.45. This was accomplished by using a venti-
latory volume of 13 to 14 ml/kg tidal vol-
une with a rate of 20/min. Cyclopropane
was administered by a nonrebreathing method.
Concentrations of cyclopropane in oxygen, as
determined by a rotatory-type flowmeter in
the anesthesia machine (Acoma), were 5, 10,
20, 40, and 60 per cent. Each was adminis-
tered for 15 to 30 min, with intervals of 30
min to two hours between successive adminis-
trations.

Another four immobilized rabbits inhaled
the same concentrations of cyclopropane con-
tinuously; the results supported the reliability
of the results obtained by intermittent
administration. The remaining five rabbits
were studied while restrained but not paralyzed.
These animals inhaled 20, 40, and 60 per cent
cyclopropane continuously, each being adminis-
tered for 15 min.

EEG activity was recorded by conventional
amplification. Multiple-unit activity was re-
corded from the midbrain reticular formation,
using the technique described in our previous
studies.\(^6\) It was obtained from the same bi-
polar electrodes which were simultaneously
recording the EEG. Electrical activity passed
from the electrode into the preamplifier of the
polygraph (Sanei 141-8). This wide-band
signal was taken out of the CRO output and
entered into a high frequency band-pass filter,
the peak frequency response of which was
centered at 1,300 Hz, with 3-dB decreases at
600 and 2,500 Hz. This high-frequency ac-


tivity was rectified and smoothed, with the
smoothing time constant of 50 msec for both
rising and falling phases (amplitude demod-
ulation). This signal consisted of fluctuation
of DC level: the higher the DC level, the greater
the firing rate of a large population of neuronal
units. These amplitude-demodulated signals
were displayed simultaneously with the EEG
(15 mm/sec paper speed) and slow oscil-
lograph (5 mm/min). The slow oscillograph
showed both a tonic basal level and fluctua-
tions: the basal level was measured as the
distance from the base of the tracing to a line
obtained by inserting a 15-kΩ resistor across
the input in place of the rabbit; the result-
ing line was considered to show the level of
system noise. Since the fluctuation did not
change significantly during the course of
the experiment, amplitude-demodulated signals
could be described by changes of basal
level. The basal level of the amplitude-de-
momulated signal and the systolic arterial pres-
sure in the control gallamine-immobilized state
served as controls in each experiment, and the
changes induced by cyclopropane were ex-
pressed by the normalized values at one-min-
ute intervals after initiation of cyclopropane
inhalation (fig. 3).

**Results**

**CNS Electrical Activities during Control
Wakefulness and Sleep**

CNS electrical activities in the unrestrained
rabbit during wakefulness and sleep were simi-
lar to those found in the cat.\(^3\) The awake
record was characterized by low-voltage fast
activity with a superimposed low-voltage theta
rhythm in the neocortical lead and rhythmic
theta waves of 5–6 Hz in the dorsal hippo-
campus (fig. 4A, a). During slow-wave sleep
both neocortical and hippocampal leads
showed irregular slow waves of large ampli-
Fig. 3. Mean changes in the basal level of reticular multiple-unit activity (●) and systolic blood pressure (▲), showing standard deviation (six rabbits). This figure shows only the changes in the initial 15 min during which each rabbit was given cyclopropane in each concentration.

- Systolic arterial blood pressure
- Basal level of reticular multiple unit activity
- Standard deviation
tude and occasional spindles (fig. 4A, b). During paradoxical sleep the hippocampal theta rhythm increased in frequency to 6-7 Hz (fig. 4A, c, d). The changes in EEG pattern in the midbrain reticular formation were not remarkable (fig. 4A, a-d).

Reticular multiple-unit activity (MUA) changed in parallel with EEG changes, i.e., it was lowest during slow-wave sleep and highest during paradoxical sleep (fig. 5, control). Arbitrarily setting at 100 per cent the basal level of reticular MUA during the awake state, it decreased by 25-30 per cent during slow-wave sleep and increased by 20-40 per cent during paradoxical sleep. Fluctuation of reticular MUA increased markedly during paradoxical sleep, but not as much during awake and slow-wave sleep.

Alerting stimuli such as the appearance of persons conducting the experiment induced an
increase in the frequency of hippocampal theta rhythm, accompanied by a 20–40 per cent increase of reticular MUA. Gallamine-immobilized non-anesthetized rabbits had EEG patterns and levels of reticular MUA similar to those of rabbits in the control awake state, which confirmed our previous observation 11 (fig. 5, 5 per cent, e, and fig. 4B, e).

**Effect of Cyclopropane on Reticular MUA in Paralyzed Rabbits**

Measurement of MUA in the midbrain reticular formation during administration of cyclopropane revealed that cyclopropane induced in the CNS a biphasic action, an initial excitation followed by a depression. Concentrations
of cyclopropane below 20 per cent induced progressive increases in the basal level of reticular MUA. Concentrations above 40 per cent induced an initial excitation, which was followed by a depression leading to a plateau. The peak value and the rate of increase of the basal level increased as the concentration was increased. With 10 per cent cyclopropane, the basal level reached a plateau in 10–15 min which was 15–20 per cent above the control level in the gallamine-immobilized state, while with 20 per cent cyclopropane the basal level reached a plateau in 6–8 min which was 20–25 per cent above control. With 40 per cent cyclopropane, the basal level showed a biphasic response, i.e., it increased initially and then decreased again. The initial increase reached a peak in 3–6 min and then reached a plateau in 10–15 min. The plateau was 10–15 per cent above control, which was lower than that induced by 20 per cent cyclopropane. Thus, the greatest activation was estimated to be induced by a concentration between 20 and 40 per cent. When 60 per cent cyclopropane was administered, reticular MUA reached its peak in 2–4 min, then a gradual decrease occurred, leading to a plateau 20–30 per cent below that of the control immobilized state in 60–90 min.

Changes in arterial blood pressure showed a trend similar to that of reticular MUA. During the gallamine-immobilized preanesthetic period, alerting stimuli such as the appearance of experimental personnel in the rabbit’s visual field, a gentle touch on the body, or blowing on the face induced an elevation in arterial pressure of 30–50 mm Hg, preceded by an abrupt increase of reticular MUA. These changes often occurred at the initiation of administration of cyclopropane because of manipulation of the ventilation system (fig. 5). Administration of cyclopropane induced a biphasic change in arterial blood pressure, i.e., an initial elevation followed by a decline. Arterial pressure and reticular MUA had different latent periods to reach the peak changes, e.g., the peak of arterial pressure elevation occurred 4–5 min after that of reticular MUA with 40 per cent cyclopropane and 2–3 min after it with 60 per cent cyclopropane (figs. 3 and 5). At the termination of inhalation of concentrations of cyclopropane greater than 20 per cent for longer than 15 min arterial pressures rose about 2–5 per cent (fig. 5). When reticular MUA increased to more than 10 per cent above the control value, mild alerting stimuli did not induce any further change in either arterial pressure or reticular MUA.

EEG PATTERNS DURING CYCLOPROPANE ADMINISTRATION

When the concentration of cyclopropane was less than 10 per cent, the cortical EEG showed a slight increase in amplitude and the dorsal hippocampus showed either rhythmic theta activity or irregular slow waves (fig. 4, f). The cortical EEG was higher in amplitude when the dorsal hippocampal EEG was irregular, and it was smaller when the dorsal hippocampus showed theta activity. Concentrations of cyclopropane above 20 per cent induced a marked change in the EEG. After the appearance of some irregular slow waves in the neocortical lead, 1–2 min in duration, 2½-Hz rhythmic slow waves (slow-wave hypersynchrony) appeared in the neocortex and 4–5-Hz high-amplitude theta rhythm in the dorsal hippocampus (fig. 4B, g). When 60 per cent cyclopropane was administered, the cortical hypersynchrony appeared only initially and then decreased in amplitude, gradually leading to an isoelectric pattern (fig. 4B, h, i). The hippocampal theta activity also decreased in amplitude, while no marked change in reticular EEG pattern was seen (fig. 4B, i).

When a very low-amplitude pattern or isoelectricity had continued for some time, high-frequency high-amplitude seizures occurred (fig. 5, 60 per cent). These EEG seizures were not accompanied by gross somatic movements or changes in arterial pressure. The details of this seizure activity will be described.25

CORRELATION OF GROSS BEHAVIOR AND CNS ELECTRICAL ACTIVITY IN NONPARALYZED RABBITS

The CNS electrical activities in nonparalyzed restrained rabbits changed in the same manner as those in paralyzed rabbits. Two to three minutes after administration of 20–40 per cent cyclopropane, when the EEG showed some slow waves, the rabbits head dropped, although it still responded to pinching by withdrawal. When hypersynchronous slow waves appeared in the neocortex, the animal lifted its head slowly and opened its eyes, and
the pupils dilated maximally. When hippocampal theta rhythm became dominant, the rabbit developed violent masticatory movements. The masticatory movements did not synchronize with either the neocortical hypersynchronous waves or the hippocampal theta rhythm. Masticatory movements occurred rhythmically at a rate of about 4/sec. When EEG depression appeared and reticular MUA decreased to the control level, the masticatory movements disappeared and the rabbit dropped its head again and became quiet. During both the masticatory movements and the succeeding quiescence, noxious stimulation such as pinching did not induce changes in EEG pattern or gross behavior, but elicited slight increases in reticular MUA.

Discussion

The basic methodology used in these studies was developed by Arduini and Finneo\textsuperscript{10} and modified by Schlag and Balvin.\textsuperscript{17} As has been discussed in our previous papers\textsuperscript{7,13} and those of others,\textsuperscript{16,17} there is little doubt that the activities obtained by this method are neuronal in origin. However, there are some limitations in the quantification of practical bioelectric measurements, which have been discussed precisely by Schlag and Balvin,\textsuperscript{17} as follows. The changes in levels of rectified activity as utilized in the present study depend upon not only the numbers but also the relative sizes of these potentials. The changes in firing rates of neuronal elements which yield large impulses are more easily reflected than those which yield smaller impulses. Another possibility is that the potentials of inverse polarities occur simultaneously and cancel each other. This would happen more easily when their rate is increased. We do not have any adequate basis for discussion of these limitations now, since there is no way to distinguish the
following factors involved: number of elements, amplitude of potentials emitted, and rate of discharges per element. Whatever the limitations mentioned above, increases in the levels of rectified activity during the transition from sleep to wakefulness\textsuperscript{15, 22} and during stimulation of various sorts\textsuperscript{18, 20, 21} have been reported.

The present study of CNS electrical activity has indicated that cyclopropane induces a biphasic CNS response: initial excitation, followed by depression. The initial state is characterized by increased firing of reticular neurons, cortical slow-wave hypersynchrony, hippocampal theta rhythm, arterial hypertension, and bizarre mastication in the rabbit. The degree of CNS excitation was related to the concentration of cyclopropane. We have described the relationship between cortical slow-wave hypersynchrony and drug-induced CNS excitation.\textsuperscript{7, 8} The rhythmic theta activity in the dorsal hippocampus, considered by Green and Arduini\textsuperscript{19} to be a sign of “hippocampal arousal,” is also indicative of the excitation of the brain-stem arousal system.

It is not clear how an anesthetic induces the opposite effects of CNS excitation and depression. It may be that there exist within the brain at least two neuronal systems, one of which is depressed by cyclopropane while the other is excited. The slower theta rhythm in the dorsal hippocampus during administration of cyclopropane might be the result of excitation of some component of the brain-stem arousal system while the other component is depressed. Unpublished data have shown that prior administration of reserpine not only blocks the excitation but reverses it to depression. Thus, cyclopropane-induced CNS excitation might be related to a metabolic process involving brain catecholamines within the CNS.

The activation of the sympathetic nervous system by cyclopropane is well known.\textsuperscript{19-21} Our previous hypothesis\textsuperscript{9} that sympathetic activation might be one of the peripheral manifestations of the brain-stem arousal system has been confirmed by the results of the present study, since arterial hypertension occurred when reticular neuronal activity was enhanced and hypotension ensued when the succeeding CNS depression appeared. Price et al.\textsuperscript{22, 23} have postulated, on the other hand, that selective depression of the medullary vasodepressor mechanisms plays a role in cyclopropane-induced sympathetic activation; this hypothesis, however, was not supported by the findings of Ngai and colleagues.\textsuperscript{22, 23} The present study also demonstrated depression of neuronal elements during deep cyclopropane anesthesia. This neuronal depression bore no relationship to the cyclopropane-induced hypertension, since it was observed when arterial pressure was normal or below control levels. Therefore, the mechanism by which cyclopropane selectively inhibits the vasodepressor mechanism, as postulated by Price et al., is still not clear.

Strong evidence\textsuperscript{19, 21, 22, 23} suggests a close functional connection of the limbic system to the brain-stem reticular core and the hypothalamus, the center of the autonomic nervous system.\textsuperscript{24} Both the arterial hypertension and the bizarre masticatory movements can be considered results of excitation of the central autonomic nervous system. That masticatory movements seen in the rabbit have not been reported to occur in man during cyclopropane anesthesia may be related to the fact that the limbic system occupies a relatively larger part of the brain in the rabbit\textsuperscript{22}; thus, the phenomenon may be evoked more easily in the rabbit.

It is widely accepted that the anesthetic state is represented by a functional and reversible block of the ascending reticular system.\textsuperscript{24-26} Eplin defined the anesthetic state as decreased excitability of the central neuronal system.\textsuperscript{24} We demonstrated previously that not only a decrease of spontaneous firing of neurons in the ascending reticular activating system but even disorganized excitation could be associated with anesthesia.\textsuperscript{7, 8} The present study has demonstrated that cyclopropane is another example of an excitatory anesthetic. It seems likely that reactive capability and the spontaneous activity of the central neurons are affected differently by different anesthetics. The relationship between these two basic activities during anesthesia will be described.\textsuperscript{30}

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References

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