PHARMACOLOGICAL STUDIES ON TRANSMISSION IN THE CENTRAL VISUAL PATHWAY IN RELATION TO EFFECTS OF PENTOBARBITAL

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Since Chang (1) demonstrated a potentiation of cortical responses to thalamic stimulation induced by continuous illumination of the retina, many investigations have been performed to elucidate the afferent tonic inhibitory effect on the central visual pathway. Arduini and Hirao (2) have shown that visually evoked responses were influenced directly by spontaneous retinal discharges. These findings have been confirmed with the following studies (3–6) which have postulated tonic suppression on the lateral geniculate neurons by means of recurrent inhibition of the geniculate relay neurons. On the other hand, other investigators (7–9) have demonstrated inhibitory effects on the geniculate neurons derived from the visual cortex. Inhibitory or facilitatory influence on the visual pathway from various brain structures, especially from the brain stem reticular formation, has also been observed by many researchers (10–15).

In previous papers (16, 17), we reported the role of reticular modulation of the visual system by observing the relation of altered visually evoked responses to associated reticular activity with some central depressants. The present experiments were designed to investigate the effects of pentobarbital on such intricate afferent transmissions through the central visual pathway and further to solve the feedback mechanism in the central nervous system.

METHODS

Experiments were performed on 25 adult cats weighing 2.5–4.0 kg. All surgery was done under diethyl ether anesthesia. After insertion of a tracheal cannula, the animal was fixed on a stereotaxic instrument. The skull over the left lateral gyrus was removed, and burr holes were made in the remaining calvarium for insertion of bipolar stimulating or recording electrodes in the ipsilateral midbrain reticular formation (RF) (2, 3, –1), optic tract (OT) (11, 7, –4), and lateral geniculate nucleus (LG) (5, 10, 4). All wound edges were infiltrated with 1% xylocaine repeatedly throughout the experiment to prevent pain and discomfort. Evoked responses in the visual cortex (VC) were recorded on the left lateral gyrus with a silver ball-tipped electrode using the nasion as an indifferent site. The location of the electrode tips was determined with the aid of a stereotaxic brain atlas of Snider and Niemer (18). After all operative procedures were completed, the animal was immobilized...
with gallamine triethiodide (5 mg/kg/hr i.v.) and respiration was maintained with an artificial respirator.

The electroretinogram (ERG) derived from the corneal surface of the right eye and the evoked responses from the OT, LG and VC to flash light stimulation (FL-s) were recorded simultaneously with a polygraph (Nihon Kohden, RM-150) and potentials were added 30 times with a computer (Sanei Sokki, Mediac MC-401). In these cases, atropine sulfate was put into the right eye for pupil dilatation. Evoked responses in the LG and VC to electrical stimulation of the ipsilateral optic tract (OT-s) were displayed on a 4 channel cathode-ray monitor (Nihon Kohden, VC-40MR) and recorded on photographic film using a long-recording camera. FL-s delivered from a photic stimulator (Nihon Kohden, MSR-1R) was applied to the right eye every 2 seconds to record ERG, and single electrical pulses to the OT were applied every 2 seconds for a 0.05 msec duration and 12 or 15 volts, which is three times the threshold. Square wave pulses of 1 msec duration to the RF were applied 50 msec prior to FL-s or OT-s with an intensity eight times above the EEG activating threshold. This intensity is found to be most effective in facilitating visual potential (17). ECG and blood pressure were monitored routinely. EEG monitoring was carried out on the left anterior sigmoid gyrus by phonograph-needles inserted into the skull. In some cases, both eyeballs were previously enucleated under local anesthesia and experiments were started more than 5 hours after operation. The animal was placed in a dark and sound-proof room maintained at 28°C, and experiments were started 30 minutes following adaptation to the dark.

Pentobarbital sodium was dissolved in 2 ml saline solution with doses of 2, 4 and 8 mg/kg and injected every 10 minutes in cumulative doses of 2, 4, 8, 16, 24 and 32 mg/kg into the radial vein cannulated with a polyethylene tube. Recording of the evoked responses was started 5 minutes after each injection as there is almost complete recovery from the

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**Fig. 1.** The effect of pentobarbital on ERG and evoked responses in the optic tract (OT) to flash light stimulation. The mean change ± S.E. in the B-wave of ERG and N, component of OT are illustrated following increasing doses of pentobarbital. The X axis represents the accumulative log dose of pentobarbital, and the Y axis the mean percent change in the amplitude of each component.

N: number of animals; Significant difference from control: *P < 0.05, **P < 0.01.
transient fall in the systemic blood pressure of each animal. At end of experiments, the animal was perfused with saline solution, and then the removed brain was fixed in ethyl alcohol for a histological examination of electrode locations. Statistical significance of the data was determined by Student's t-test.

**FIG. 2.** Modification of evoked responses in the optic tract (OT), lateral geniculate nucleus (LG) and visual cortex (VC) to flash light stimulation before and after conditioned reticular stimulation (RFs) by increasing doses of pentobarbital.

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RESULTS

Pentobarbital on ERG, and evoked responses in the OT, LG and VC to FL-s

In the recording of the ERG induced by flash light with 1 msec duration in the present experiment, only the B-wave was found. As shown in the left panel of Fig. 1, intravenous administration of pentobarbital did not cause any significant changes in the B-wave of ERG, though the increased tendency of the amplitude was visible in doses above 8 mg/kg. Three or four negative waves in the evoked potentials of the OT were usually observed in response to FL-s, as illustrated in Fig. 2. The first negative component (N₁) appeared in the latency of 13.8 to 17.5 msec. As can be seen in the right panel of Fig. 1, the amplitude of N₁ gradually decreased in accordance with increased amount of pentobarbital and the amplitudes at doses of 2 and 4 mg/kg were 93.6 and 90.3% of control respectively. At doses of 8, 16 and 32 mg/kg, the N₁ was significantly depressed and the amplitudes were 84.3, 77.2 and 57.7% of control, respectively.

Evoked potentials of the LG in response to FL-s consist of first negative component (N₁) with 13.9 to 18.8 msec latency followed with positive waves (see Fig. 2). The left panel of Fig. 3 shows effects of pentobarbital on N₁ and the following first positive component (P₁). The N₁ resulted in an increasing tendency of amplitude, and a significant increase of 116.9% of control was seen at 4 mg/kg. At doses of 24 and 32 mg/kg, significant increases in amplitude were recorded at 121.2 and 126.7% respectively. No significant change was found in the amplitude of P₁ after doses of pentobarbital.

![Fig. 3](Image)

Fig. 3. The effect of pentobarbital on evoked responses in the lateral geniculate nucleus (LG) and visual cortex (VC) to flash light stimulation. The mean changes ± S.E. in the N₁ and P₁ components of LG, and in the P₁ and N₁ components of VC are illustrated following increasing doses of pentobarbital.

N₁ : the first negative component; P₁ : the first positive component. N : number of animals; Significant difference from control : *P<0.05, **P<0.01.
There were the first positive component (P₁) of 20.1 msec average latency and several negative peaks following P₁ in the evoked potentials of the VC in response to FL-s (see Fig. 2). The injection of pentobarbital caused progressive decrease in the amplitude of P₁ and the first negative component (N₁), as can be seen in the right panel of Fig. 3. The amplitude of P₁ at a dose of 4 mg/kg was 80.5% of control and it was 62.2, 41.1 and 20.2% of control at 8, 16 and 32 mg/kg, respectively. These values were all statistically significant.

_Pentobarbital on evoked responses in the LG and VC to OT-s before and after enucleation of both eyeballs_

As shown in Fig. 4, apparent changes in amplitude of the evoked potentials in the LG in response to OT-s did not occur with injection of pentobarbital up to 32 mg/kg in cases of intact eyes, with the exception of a slight increase in amplitude of tract (t) component above 16 mg/kg. On the other hand, evoked responses of the LG to OT-s in cases of enucleated eyeballs resulted in a progressive decrease in amplitude, the decrease being slight in t component and more apparent in radiation (r) component accompanied by an increased amount of pentobarbital.

**Fig. 4.** Effects of pentobarbital on evoked responses in the lateral geniculate nucleus to ipsilateral optic tract stimulation in cases of intact eyes and after enucleation of both eyeballs. The mean changes ± S.E. in the tract component (t) and radiation component (r) are illustrated following increasing doses of pentobarbital.

N : number of animals ; Significant difference from control : *P<0.05.

Effects of conditioned RF stimulation on the evoked responses in the LG to OT-s were facilitatory on the amplitude of r component as previously reported (16). In the present experiment, the amplitude of r component with RF stimulation was 121.5% of control. The reticular facilitation on r component was abolished by pentobarbital in doses above 8 mg/kg in cases of intact eyes. In contrast, the facilitatory response to RF stimulation diminished after enucleation (Table 1).

The effects of pentobarbital on the evoked responses of the VC to OT-s in cases of intact eyes have already been reported (17). In cases of enucleation, however, increasing doses of pentobarbital resulted in a progressive decrease in amplitude of each component of the
evoked potentials in the VC, as shown in Table 2. The latencies of evoked potentials to FL-s in the OT, LG and VC were gradually enlarged after administration above 4 mg/kg of the drug. The latency of 17.5 msec initially in the N component of the OT response was prolonged to 18.8, 23.0 and 27.5 msec at doses of 4, 16 and 32 mg/kg, respectively. In the P1 component of VC, the latency of 20.1 msec before the drug was changed to 21.9, 23.8 and 26.3 msec at doses of 4, 16 and 32 mg/kg, respectively. However, almost no change was observed in the latencies of evoked potentials to OT-s in the LG and VC after the injection of pentobarbital.

**TABLE 1. Pentobarbital on reticular modulation of evoked responses in LG to optic tract stimulation before and after enucleation of both eyeballs.**

| Intact eyes | Enucleation of eyeballs |
|-------------|-------------------------|
| No. of animals | Tract | Radiation | Tract | Radiation |
| 0 | 97.4 ± 1.0 | 121.5 ± 2.9* | 98.2 ± 2.3 | 99.0 ± 3.1 |
| 2 mg/kg | 101.5 ± 1.0 | 122.6 ± 3.6* | 107.4 ± 7.0 | 100.2 ± 3.9 |
| 4 » | 100.1 ± 0.7 | 118.6 ± 2.8* | 102.0 ± 0.6 | 103.8 ± 1.7 |
| 8 » | 98.4 ± 0.7 | 111.9 ± 3.7 | 98.2 ± 1.3 | 103.0 ± 5.7 |
| 16 » | 100.0 ± 1.0 | 104.0 ± 1.5 | 101.0 ± 0.6 | 106.5 ± 3.9 |
| 32 » | 101.4 ± 2.1 | 101.0 ± 0.6 | 100.2 ± 0.2 | 100.4 ± 0.4 |

Each figure represents RFs/RFs x 100%, Mean ± standard error.
Significant increase : +P<0.05.

**TABLE 2. Pentobarbital on evoked responses in VC to optic tract stimulation after enucleation of both eyeballs.**

| No. of animals | P1 | P2 | P3 | N |
|----------------|----|----|----|---|
| 0 | 100% | 100% | 100% | 100% |
| 2 mg/kg | 89.1 ± 9.5 | 89.7 ± 15.4 | 76.1 ± 17.7 | 86.7 ± 19.6 |
| 4 » | 83.2 ± 11.2 | 80.0 ± 13.7 | 71.4 ± 18.0 | 77.5 ± 23.2 |
| 8 » | 87.2 ± 12.1 | 78.4 ± 14.3 | 64.9 ± 16.5 | 70.0 ± 21.6 |
| 16 » | 72.3 ± 17.6 | 70.8 ± 19.6 | 56.4 ± 16.9 | 65.5 ± 27.2 |
| 32 » | 63.5 ± 16.8 | 67.5 ± 22.5 | 37.2 ± 12.4* | 44.4 ± 22.2 |

Mean ± standard error. Significant decrease : *P<0.05.

evoked potentials in the VC, as shown in Table 2.

The latencies of evoked potentials to FL-s in the OT, LG and VC were gradually enlarged after administration above 4 mg/kg of the drug. The latency of 17.5 msec initially in the N1 component of the OT response was prolonged to 18.8, 23.0 and 27.5 msec at doses of 4, 16 and 32 mg/kg, respectively. In the P1 component of VC, the latency of 20.1 msec before the drug was changed to 21.9, 23.8 and 26.3 msec at doses of 4, 16 and 32 mg/kg, respectively. However, almost no change was observed in the latencies of evoked potentials to OT-s in the LG and VC after the injection of pentobarbital.

*Pentobarbital on reticular modulation of visually evoked responses to FL-s*

It has been reported by several investigators that there are no efferent fibers to the retina through the optic nerve (19-21). In this experiment, conditioned RF stimulation did not produce any changes in ERG and/or in the evoked responses of the OT to FL-s. These observations gave support to the argument mentioned-above. No significant change was observed in the evoked responses of the LG to FL-s after RF stimulation, as shown in Table 3. The conditioned RF stimulation produced depressive or facilitatory effects on the evoked potentials in the VC. Facilitation in amplitude of cortically evoked
potentials to FL-s induced by conditioned RF stimulation was usually observed in cases showing resting pattern in background EEG. On the other hand, inhibitory influence appeared mostly in cases of arousal pattern in EEG. Table 4 shows the effects of pentobarbital in 6 cases of depression and 3 cases of facilitation after RF stimulation. In the former cases, amplitudes of the P and N of evoked responses in the VC to FL-s were decreased to 80.9 and 85.3% of control, respectively, following RF stimulation. After injection of pentobarbital, depression of the P and N diminished and the amplitudes increased higher than control with RF stimulation at total doses above 8 mg/kg. In the latter cases, facilitatory effects induced by RF stimulation also disappeared at 2 mg/kg of pentobarbital.

\[ \text{Table 3. Pentobarbital on reticular modulation of ERG, and of evoked responses in OT and LG to flash light stimulation.} \]

| No. of animals | ERG | Optic tract | Lateral geniculate body |
|----------------|-----|-------------|------------------------|
|                | 6   | 8           | 8                      |
| 0              | 101.1±0.6 | 93.5±1.8 | 90.1±5.4 | 104.3±3.5 |
| 2 mg/kg        | 99.8±0.6 | 98.1±2.1 | 91.4±6.1 | 110.3±4.0 |
| 4              | 100.2±1.1 | 95.4±1.5 | 87.9±2.8 | 105.4±3.9 |
| 8              | 97.2±0.9 | 104.4±6.9 | 109.4±5.3 | 108.2±2.6 |
| 16             | 98.0±1.6 | 106.4±2.1 | 97.6±2.9 | 106.5±2.6 |
| 24             | 92.9±2.0 | 100.1±4.8 | 95.0±1.9 | 101.2±2.7 |
| 32             | 91.1±5.5 | 102.7±2.7 | 98.4±3.6 | 101.2±2.2 |

Each figure represents RFs ×100%, Mean±standard error.

\[ \text{Table 4. Pentobarbital on reticular modulation of evoked responses in VC to flash light stimulation.} \]

| No. of animals | Depressed cases | Facilitated cases |
|----------------|-----------------|-------------------|
|                | Positive        | Negative          | Positive | Negative |
|                | 6               | 6                  | 3        | 3         |
| 0              | 80.9±5.5**      | 85.3±6.2*         | 133.0±1.7** | 126.2±15.2 |
| 2 mg/kg        | 86.1±6.6        | 101.4±8.6         | 101.5±7.4 | 101.6±4.6 |
| 4              | 117.5±7.3       | 90.6±5.8          | 93.8±8.7 | 106.0±1.1 |
| 8              | 123.5±3.1       | 97.8±7.0          | 124.4±10.4 | 119.1±16.2 |
| 16             | 128.5±4.1       | 105.4±10.7        | 102.2±12.5 | 104.8±10.5 |
| 24             | 130.0±10.2      | 103.1±8.5         | 68.8±14.4 | 102.6±9.7 |
| 32             | 116.7±11.4      | 97.9±6.5          | 91.7±12.7 | 91.7±12.1 |

Each figure represents RFs ×100%, Mean±standard error.

Significant increase : **P<0.01.
Significant decrease : *P<0.05, **P<0.01.
To date, there has been no direct evidence of a recorded electrical activity of the bipolar cells in the retina. The B-wave of ERG is considered to be derived probably from activity in the inner nuclear or ganglionic layer. Table 5 shows effects of pentobarbital on visually evoked responses to FL-s and OT-s in cases of intact eyes and after enucleation of both eyeballs. Effects of pentobarbital on the inner nuclear layer were negligible due to lack of influence on the B-wave by injection up to 32 mg/kg of the drug. Evoked responses in the OT to FL-s show the activity originating in axons of the ganglion cells in the retina. A few negative waves in these responses are likely to be derived from several different kinds of fibers which have respectively different conduction velocities as pointed out by Bishop et al. (22) and Lennox (23). Evoked responses in the OT to FL-s were gradually depressed in accordance with increasing doses of pentobarbital. It can be said that the drug produces some inhibitory effects on the ganglion cells though pentobarbital have not exerted complete depression on the retina as explained in a further report.

In marked contrast, evoked responses in the LG to FL-s were not depressed by pentobarbital, and amplitude of the N1 component was increased higher than control with larger doses. Furthermore, pentobarbital caused almost no change in evoked responses of the LG to OT-s in cases of intact eyes. After enucleation, r component of the evoked responses in the LG to OT-s was gradually depressed in accordance with increasing doses of pentobarbital.
Facilitatory as well as inhibitory effects on the LG neurons from the retina, visual cortex and/or reticular formation etc. has been widely studied. Tonic inhibitory influence on the central visual system derived from the retina has been demonstrated by several investigators (2-6, 24). Disinhibition of this influence by means of a continuous illumination of the retina or temporary high ocular pressure or enucleation of both eyeballs resulted in a marked decrease of spontaneous discharges and in an apparent increase in amplitude of postsynaptic component of the field potentials in the LG neurons. Suzuki et al. (3, 6) have shown that the suppressed state presented in the relay cells of the LG was due to postsynaptic inhibitory action produced through the interneuron by optic nerve volleys. Furthermore, it has been reported by Iwama et al. (7) and Angel et al. (9) that the LG neurons were given inhibitory influence from the VC. It has been presumed that deafferentation on the retina followed by the decrease of ascending impulses to the LG and the silence of inhibitory interneurons in the LG and depressive effect on the VC produces elevated excitability in the OT fibers and the LG neurons and these, in turn, cause increases in amplitude of the evoked responses in the LG to OT-s.

In the present experiments on cases of intact eyes, the negligible effects of pentobarbital on the LG neurons could be due to the disinhibition to the LG neuron following the depressant action of the drug on the ganglion cells of the retina and the visual cortex. However, direct depressant effects of pentobarbital on the LG should be pointed out as there is progressive decrease in amplitude of evoked responses in the LG to OT-s after enucleation. These results suggest that effects of pentobarbital on the LG will appear in balance between direct inhibitory action on the LG neurons and disinhibitory phenomenon of the LG produced by depressant effect on the retina and the visual cortex. This assumption also supports our previous report (17) in which a dramatic increase in amplitude of P component in the VC at a dose of 32 mg/kg of pentobarbital was demonstrated. It can be concluded that effects of pentobarbital on the visually evoked responses may appear in relation to direct action on a specific area and to indirect action on the feedback system derived from related areas in the central nervous system.

After administration of pentobarbital, the latencies of evoked potentials to FL-s in the OT, LG and VC were gradually prolonged, however, any prolongation in latencies of evoked potentials to OT-s in the LG and VC were not observed. These findings show that the time consumption in the prolonged latency of evoked response to FL-s after injection of pentobarbital resulted in the delayed synaptic transmission within the retina.

Both inhibitory and facilitatory influences were observed on the evoked responses to FL-s in the VC after conditioned RF stimulation, while little effect of the RF on the evoked responses to FL-s in the OT and LG was seen. Pentobarbital was more effective to the facilitatory modulation by RF stimulation than that of the inhibitory. The reverse phenomenon to the facilitation on the cortical responses to FL-s following conditioned RF stimulation at small doses of pentobarbital could result in a change in background activity of EEG. However, we have not been able to find out the reason at this time.
SUMMARY

Effects of pentobarbital on ERG, and on evoked responses in the optic tract (OT), lateral geniculate nucleus (LG) and visual cortex (VC) induced by flash light to the contralateral eye were studied in cats. Effects of pentobarbital on evoked responses in LG and VC to OT stimulation were compared between cases of intact eyes and those in which both eyeballs were enucleated. Pentobarbital caused no significant change in ERG, but depressed evoked responses to flash light in OT. Responses evoked to flash light in LG showed an increase in the first negative component, but the cortical potentials were progressively depressed by pentobarbital. The drug caused a gradual decrease in the post-synaptic component of evoked responses in LG to OT stimulation in cases of enucleation, though no significant change was observed in evoked responses of LG with intact eyes. Inhibitory or facilitatory modulation of visually evoked responses induced by conditioned stimulation of the reticular formation disappeared after administration of pentobarbital. Effects of pentobarbital on the visually evoked responses may appear in relation to direct action on a specific area and to indirect action on the feedback system derived from related areas in the central nervous system.

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