EFFECT OF 5-HYDROXYTRYPTAMINE INJECTED LOCALLY INTO THE BRAIN STEM ON EEG AND ECG IN CHICKENS

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Effects of 5-hydroxytryptamine (5-HT) on the central nervous system (CNS) have been studied extensively. Of them, the effect on the cerebral-electrical activity of animals has been reported. MASHKOVSKII and ROSHCHINA (1962) observed in cats that high-voltage slow waves appeared in both the visual and motor areas of the cerebral cortex at a dose of 1~2 mg/kg of 5-HT injected intravenously. ROTHBALLER (1957) noticed an activation followed by a deactivation in cats administered with 5~30 μ g of 5-HT by the intracarotid route. KOELLA and CZICMAN (1966) reported that the intracarotid injection of 5-HT (0.2~5.0 μ g/kg) in cats had induced initially an arousal pattern lasting for 30~150 seconds, and that this pattern had been replaced later by a protracted phase of hypersynchrony often lasting as long as 15 minutes. The same biphasic effect of 5-HT was also described by MONNIER and TISSOT (1958) in rabbits. VOGT, GUNN and SAWYER (1957) found that intraventricular injection of 5-HT (750 μ g) in curarized cats decreased the electrical activity of all the regions of the brain, except the bulbar reticular formation in which long, spindle-like bursts rose to 45 Hz. SPOONER and WINTERS (1965) observed that this compound (0.1~20 mg/kg) administered subcutaneously produced depressant effects on the electroencephalogram (EEG) and behavior in chicks (5 to 14 days old). HEHMAN, VONDERAHE and PETERS (1961) injected newly hatched chicks subcutaneously with varying doses of 5-HT and obtained essentially the same results as shown by SPOONER and WINTERS (1965) when small doses had been used, but observed an activating effect when large doses had been injected.

These contradictory results might be due not only to the difference in dose of 5-HT, but also to the differences in species of the animal used and in the state of development of the CNS. Moreover, the effects of 5-HT on the CNS might also be influenced by the route of administration of 5-HT, because it seems difficult for the amine to pass readily through the blood-brain barrier of the CNS (COSTA and APRISON, 1958). Then, if 5-HT is injected systemically, it will display such direct actions as those causing changes in respiration, blood pressure, blood flow in the brain, blood sugar level, and body temperature.

The present investigation was performed to ascertain what changes would be induced in the EEG and the electrocardiogram (ECG) of chickens by 5-HT injected locally to the brain stem, especially the hypothalamus and adjacent structures.

MATERIALS AND METHODS

Experiments were carried out with 18 male White Leghorn chickens 9~12 weeks of age, weighing from 800 to 1,500 g. In these birds, EEG-recording electrodes and a

stainless-steel cannula (about 1 mm in diameter) were implanted chronically at several sites of the brain and fixed on the skull with dental resin. EEGs were recorded from the surface of the dura mater, the corpus striatum (hyperstriatum accessorium), and the anterior hypothalamic area by unipolar lead. The comb was chosen as the indifferent point. The cannula was implanted either at the anterior or posterior hypothalamus or at the posterior thalamus, so that it might be used for injection of 5-HT, noradrenaline, and saline by means of a microsyringe. ECG was recorded from the skin at the musculus cephalicus to which needle electrodes had been applied.

At termination of the experiment, 25 V direct current was run to the sites of the electrodes and cannula for 3 seconds. Then, the chicken used was killed by decapitation, and the brain removed rapidly from it. The isolated brain was fixed in 15% solution of formalin in physiological saline solution. Then, it was sliced into sections 30 μ in thickness by the freezing technique and stained with cresyl-violet. The exact position of the cannula tip, as well as the electrode sites, was ascertained histologically.

Creatinine sulfate of 5-HT was dissolved in saline (1 mg/ml). Before the experiment was carried out, it was confirmed that no saline injected into any part of the brain exerted influence either on EEG or on ECG.

RESULTS

Figure 1 gives the results obtained from one of the representative cases injected with a dose of 10 μ l of 5-HT into the right posterior hypothalamus. Before the injection, the surface EEG had shown high-voltage slow waves of 80~100 μ V and 4~6 Hz. Concomitant with this time, the bird closed both eyes (Fig. 1, A). About 2 minutes after the injection, low-voltage fast waves of 20~30 μ V and 20~30 Hz were observed on the surface EEG led from the right side of the brain (Fig. 1, B). These waves lasted for about 4 minutes. Although the bird closed both eyes, the waves seemed to be essentially the same as those appearing at the time of wakefulness. Six to eight minutes

Fig. 1. Effect of 5-hydroxytryptamine (5-HT) on EEG and ECG of the Chicken

Section A indicates the results obtained before the experiment was carried out. Sections B, C, and D indicate the results obtained 2, 8, and 15 minutes, respectively, after injection of 10 μ l of 5-HT into the posterior hypothalamus. R: SUR. 1 and R: SUR. 2 indicate two sites of the surface on the right side, and L: SUR. 1 and L: SUR. 2 two sites of the surface on the left side. ECG indicates the electrocardiogram. Scale: 1 second. 100 μ V.

Fig. 2. Position of the Tip (Arrow) of a Cannula Implanted in the Posterior hypothalamus

Fig. 3. Effect of 5-HT on EEG and ECG of the Chicken

Sections A, B, C, and D indicate the results obtained before, and 2, 8, and 15 minutes after injection of $10\mu\text{l}$ of 5-HT into the posterior dorsal thalamus, respectively. R: HA and L: HA indicate EEGs led from the hyperstriatum accessorium of the brain on the right and the left side, respectively. R: SUR and L: SUR indicate the right and the left side surface EEG, respectively. ECG indicates the electrocardiogram. Scale: 1 second. $100\mu\text{V}$.

thereafter, high-voltage slow waves of $80\sim 100\mu\text{V}$ and $1\sim 3\text{ Hz}$ occurred, accompanying the fast waves as described above (Fig. 1, C). Finally, they showed a significant synchronization; that is, high-voltage slow waves of $100\sim 120\mu\text{V}$ and $2\sim 3\text{ Hz}$ (Fig. 1, D) exhibited a synchronization which continued for about 60 minutes. Then, the EEG returned to such normal pattern as shown before the injection. In contrast to these changes in EEG recorded from the right side of the brain, not a change occurred to the left surface EEG or ECG obtained after central administration with 5-HT. It was demonstrated histologically that the cannula tip had been located at a site adjacent to the nucleus hypothalamicus lateralis of the brain on the right side (Fig. 2).

Figure 3 shows one of the representative patterns obtained after the injection with a dose of $10\mu\text{l}$ of 5-HT into the right posterior dorsal and ventral thalamus. The surface EEG changed in essentially the same manner as shown in Fig. 1. Moreover, an EEG led from the corpus striatum or the anterior hypothalamus revealed a synchronization with the surface EEG. On the other hand, a decrease appeared in heart rate. In this case, changes occurred within 30 seconds after the injection and lasted for about 8 minutes. After these early changes, the heart rate tended to increase gradually and returned eventually to the initial level about 26 minutes after the injection. When

Fig. 4. Effect of Noradrenaline on EEG and ECG of the Chicken

Sections A, B, and C indicate the results obtained before, and 2 and 8 minutes after injection of $10\mu\text{l}$ of noradrenaline into the posterior dorsal thalamus, respectively. R: SUR and L: SUR indicate the right and the left side surface EEG, respectively. ECG indicates the electrocardiogram. Scale: 1 second. $100\mu\text{V}$.

5-HT was injected into the anterior hypothalamus or mesencephalon, such influence as described above was observed neither on EEG nor on ECG.

When a dose of $10\mu\text{l}$ of noradrenaline (1 mg/ml) was injected into the anterior and posterior hypothalamus, neither EEG nor ECG was influenced with it. On the contrary, when the same dose of noradrenaline was injected into the posterior dorsal and ventral thalamus, a decrease appeared in heart rate, but no EEG was influenced at all (Fig. 4).

The 18 birds used for this experiment were examined histologically to ascertain the sites where the tips of the cannulae had been located. These tips were in the dorsal and ventral thalamus, including the nucleus ovoidalis, nucleus rotundus, and nucleus entopeduncularis ventralis superior, in 8 birds, the posterior hypothalamus, including the nucleus hypothalamicus lateralis and the lateral forebrain bundle, in 6 birds, the anterior hypothalamus, including the nucleus hypothalamicus anterior medialis, in 2 birds, and the mesencephalon, including the nucleus ruber and the pars ventralis of the nucleus mesencephalicus profundus, in 2 birds.

DISCUSSION

The results of this investigation show that 5-HT injected into the posterior hypothalamus and the posterior dorsal and ventral thalamus induced an initial arousal-EEG pattern, which was replaced secondarily by a protracted phase of hypersynchrony 6~8 minutes thereafter. These slow waves lasted for about 60 minutes. The heart rate decreased markedly only when 5-HT had been injected into the thalamus. On the other hand, when this compound was injected into either the anterior hypothalamus or the mesencephalon, EEG and ECG were free from its influence. These results suggest that only the posterior hypothalamus and the posterior thalamus may have sensitivity for 5-HT. In mammals, the hypothalamus and adjacent structures usually have the highest concentration of 5-HT (BERTHER and ROSENGREN, 1954). In avian species, the hypothalamus and adjacent structures contain 5-HT at a higher level than the other parts of the brain, except the cerebral hemispheres (PSCHIEDT and HIMWICH, 1963; JUORIO and VOGT, 1967). No change of EEG induced by 5-HT can be regarded as any secondary effect which may have been caused by an inadequate blood supply to the brain. When injected into the posterior dorsal and ventral thalamus, 5-HT and noradrenaline caused initially a decrease in heart rate. EEGs, however, were influenced by 5-HT but not by noradrenaline.

A biphasic action of 5-HT on EEG has been reported by ROTHBALLER (1957), VOGT, GUNN and SAWYER (1957), and KOELLA and CZICMAN (1966) in cats, and by MONNIER and TISSOT (1958) in rabbits. Among them, KOELLA and CZICMAN (1966) established the following hypothesis: The EEG-desynchronizing action might be attributed to the anterior brain stem or the forebrain, and the EEG-synchronizing action to the lower brain stem. The lower brain stem included the area postrema, which might contain such receptors as causing EEG-synchronization after injection with 5-HT. KOELLA and CZICMAN (1966) did not succeed, however, in pointing out any corresponding receptor area in the anterior brain stem or in the forebrain.

From the present investigation, the authors establish no hypothesis concerning the EEG-desynchronization or EEG-synchronization induced by 5-HT. It is presumed that a receptor related to both EEG phenomena might be included in the posterior thalamus and the posterior hypothalamus, because 5-HT injected locally into such regions as described above seemed to diffuse within a sphere about 1 mm in diameter.

SUMMARY

The action of 5-hydroxytryptamine (5-HT) injected locally into the hypothalamus and adjacent structures was studied electrographically. The following results were obtained.

(1) When 5-HT (10~30 μ l) was injected into the posterior dorsal and ventral thalamus or the posterior hypothalamus, the desynchronization of EEG appeared initially in each unilateral region about 2 minutes after the injection. About 6 minutes thereafter the desynchronization was followed by the synchronization or the appearance of slow waves. The latter lasted for about 60 minutes.

(2) When 5-HT was injected into the posterior dorsal and ventral thalamus, there was a decrease in heart rate. The decrease occurred within 30 seconds after the injection and lasted for 8 minutes or more. Then, the heart rate tended to increase conversely and returned to the original level about 26 minutes after the injection.

(3) The effect of 5-HT induced on EEG could not be regarded as the indirect action of this compound, although the compound caused an inadequate blood supply to the brain.

(4) Since 5-HT injected locally into the posterior hypothalamus and the posterior dorsal and ventral thalamus seemed to diffuse within a sphere about 1 mm in diameter, a receptor concerning the biphasic action of EEG induced by 5-HT might be included in the regions described above.

REFERENCES

- 1) AMIN, A.H., CRAWFORD, T.B.B. and GADDUM, J.H. (1954): *J. Physiol. (Lond.)*, **126**, 596~618.
- 2) BERTHER, A. and ROSENGREN, E. (1959): *Experientia. (Basel)*, **15**, 10~11.
- 3) COSTA, E. and APRISON, M.H. (1958): *Amer. J. Physiol.*, **192**, 95~100.
- 4) HEHMAN, K.N., VONDERAHE, A.R. and PETERS, J.J. (1961): *Neurology. (Minneap.)*, **11**, 1011~1016.
- 5) JUORIO, A.V. and VOGT, M. (1967): *J. Physiol. (Lond.)*, **189**, 489~518.
- 6) KOELLA, W.P. and CZICMAN, J. (1966): *Amer. J. Physiol.*, **211**, 926~934.
- 7) MASHKOVSKI, M.D. and ROSHCINA, L.F. (1964): *Fed. Proc.*, **23**, 885~889.
- 8) MONNIER, M. et TISSOT, R. (1958): *Helv. physiol. pharmacol. Acta*, **16**, 255~267.
- 9) PSCHIEDT, G. R. and HIHWICH, H. E. (1963): *Life Sci.*, **7**, 524~526.
- 10) ROTHBALLER, A.B. (1957): *Electroenceph. clin. Neurophysiol.*, **169**, 409~417.
- 11) SPOONER, C.E. and WINTERS, W.D. (1965): *Experientia. (Basel)*, **21**, 256~258.
- 12) VOGT, M., GUNN, C.G. and SAWYER, C.H. (1957): *Neurology. (Minneap.)*, **7**, 559~566.

脳幹内局所注入の 5-hydroxytryptamine のニワトリの 脳波・心電図に及ぼす作用

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著者らは、ニワトリの脳幹内に局所的に注入した 5-hydroxytryptamine (5-HT; serotonin) が、脳波および心電図にどのような作用を及ぼすかを検討し、次の結果を得た。

1) 5-HT (10~30 μ l) を後部視床背、腹側部領域および後部視床下部領域に注入すると、2分後に脳波の非同期化 (20~30 μ V, 20~30 Hz) が、まず注入側に現われ、6~8分後に高振幅波 (80~100 μ V, 1~3 Hz) が、上述の非同期の波に混入し、次いで、高振幅、徐波 (100~120 μ V, 2~3 Hz) が現われ、60分前後継続して、注入前の脳波にもどった。一方、反対側の脳波では、このような変化は見られなかった。

2) 5-HT を後部視床背、腹側部領域に注入すると、心搏動数の著しい減少が、30秒以内に起こった。この減少は8分以上継続して、その後次第に増加し、26分後には、注入前の心搏動数にもどった。

3) 5-HT が脳波に及ぼす影響は、脳への血液供給の不足を起こす間接的な作用の結果とは考えられない。

4) 後部視床背・腹側部領域および後部視床下部領域に注入された 5-HT は、約 1 mm³ の球状に拡散することから考えると、上記の脳波の二相性の変化に関与する受容器は、これらの領域に存在するものと思われる。