The Waking Action of Histamine

Our recent experiments on the humoral transmission of sleep and wakefulness showed that the dialysate from sleeping and waking rabbits contains histamine in different concentrations (MONNIER[1]). This suggests that histamine may play a role in the regulation of wakefulness.

Little experimental work has been devoted to the action of histamine on the brain, and a possible involvement of this substance in the regulation of wakefulness has never been seriously considered. This is surprising since some biochemists have detected a high concentration of histamine in the hypothalamus (HARRIS et al.[2]; CROSSLAND[3]; WHITE[4]). Furthermore, it was reported that i.v. injection of histamine in the rat induces a prolonged desynchronization of the electrical brain activity, symptomatic of arousal, with a short blood pressure rise preceding a subsequent fall (BOVET et al.[5]). A similar brain activation after i.v. injection was described in the rabbit (GOLDSTEIN et al.[6]). An activating effect was also observed in the cerebellum following intraarterial injection (CROSSLAND and MITCHELL[7]; CROSSLAND[3]).

Since the effects of a single injection or of repeated single injections of histamine on the brain are of limited duration, and often masked by the much stronger waking action of the human operator (GANGLOFF and MONNIER[8]), we adopted for our present investigations the technique of constant flow infusion with a perfusion pump. This allowed the elimination of factors of error inherent to human presence and irregular injection flow.

Methods. Our experiments were performed on 16 conscious rabbits, weighing 2.6–3.0 kg. The holes in the skull for the recording electrodes were bored the day before. The electrical activity was recorded from the motor and sensory-motor cortex with dural silver electrodes screwed into the skull and from the hippocampus with platinum needle electrodes, which were all insulated except at the tip. For accurate localization of the structures and placement of the electrodes, we used the stereotaxic method of MONNIER and GANGLOFF[9].

We cannulated the femoral artery under local anaesthesia for recording the blood pressure and the external jugular vein for injection of histamine or control fluid. We also recorded the heart activity with an ECG and the respiration with a thermocouple connected to a channel of the amplifying apparatus.

All these cerebral and visceral activities were recorded with a 16-channel electroencephalograph (Schwarzer). Simultaneously, the electrical activity of the motor cortex and hippocampus was analysed with an automatic frequency analyser (Faraday Electrical Instrument Co.). For this purpose, the animals were immobilized in a hammock and kept in a sound-proof dark-shielded cage.

The infusion of histamine-dihydrochloride into the external jugular vein was performed with a perfusion pump (Braun, Melsungen) at a constant flow rate of 1ml/min for 30 min. Histamine-dihydrochloride was dissolved at a concentration of 1 mg/30 ml and kept at pH 7.4. We infused the whole amount of this solution. As control fluid, we used a dialysing fluid which had proved quite suitable in our previous experiments on hemodialysis (MONNIER, HÖSLI, and KOLLER[10]).

The best parameter of sleep is the high voltage slow delta activity of the motor cortex (0.5–3.5 c/sec), recorded with and EEG and quantitatively evaluated with the automatic frequency analyser. When sleep decreases and the animal becomes alert, the amount of delta activity decreases markedly. As basic initial value, we took the arithmetic mean (= 0%) of the delta activity of 8 histamine and 8 control experiments during a preliminary period of 20 min. The deviation (in %) from this mean delta value which occurred during and after the infusion was measured and plotted upon the ordinate of a diagram (Figure 1). On the abscissa, we reported the time divided into periods of 10 min. An increase or decrease of the delta % from the basic value was indicated by + or −. Finally, we compared with the *t*-test the changes occurring during the infusion of histamine with those of the control experiments. A variation was considered as significant when its probability of error was smaller than 0.05 ($p < 0.05$).

We performed a similar analysis and diagrammatical presentation for the visceral effects: blood pressure, heart and respiration rates (Figure 2). We always took into account the cerebral and visceral phenomena of a preliminary control period of 20 min, an infusion period of 30 min and a post-infusion period of 30 min.

Results. The cortical delta activity sharply decreases with the onset of the histamine infusion and reaches half of its initial value after 15 min (Figure 1). This decrease, symptomatic of arousal, becomes already significant after 5 min, compared to the basic value and proceeds during the whole infusion. Within 30 min after the infusion, the delta activity remains at about the same low level. Concurrently with the delta decrease, the periods of desynchronization of the EEG increase in number and duration during and after the infusion. Since these

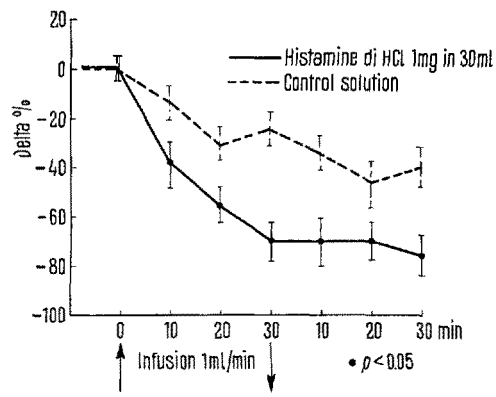

Fig. 1. Variation in % of the cortical delta activity during and after i.v. injection of histamine solution and control solution in the rabbit (mean values of 8 rabbits in each case).

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manifestations of arousal occurred during and after the infusion as well, they cannot be attributed to some rebound effect.

Control infusions of dialysing fluid also show a gradual decrease of the cortical delta activity. This becomes statistically significant after 10 min. After the infusion, a further small decrease is still detectable for 20 min.

The comparison of the effect of histamine solution and control fluid reveals a much steeper decrease during the infusion of histamine. The difference is significant from the 8th min throughout the experiment.

The visceral effects of histamine infusion are the following under our experimental conditions: (1) Slight transitory decrease of blood pressure, but not significant when compared to the control experiment. (2) Increase of pulse rate during the infusion, significant after 10 min; after the infusion, the pulse rate gradually slows down. (3) The respiratory rate has a tendency to rise, but chiefly after the infusion.

These findings suggest that histamine has a waking action independant of the slight unsignificant visceral alterations and that it could play a role in the regulation of wakefulness[11].

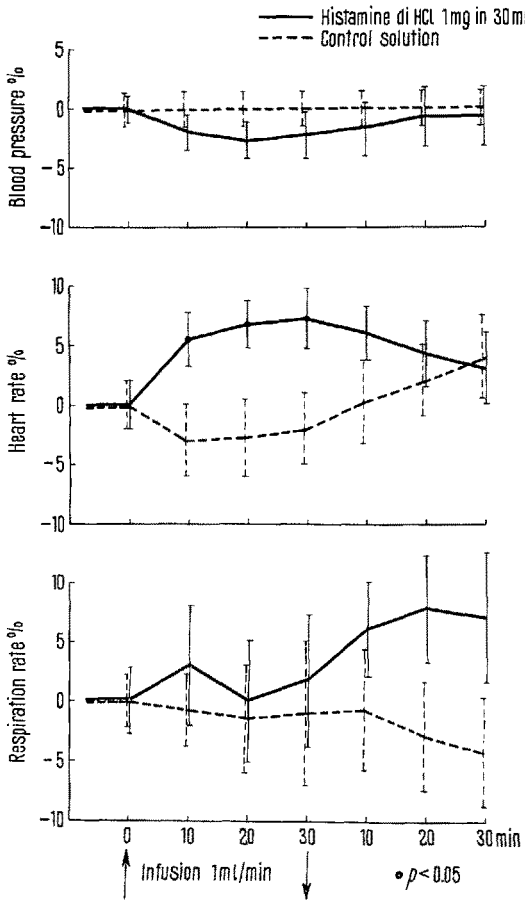

Fig. 2. Variation of blood pressure, heart rate and respiration rate in % during and after i.v. injection of Histamine solution or control solution in the rabbit (mean values of 8 rabbits in each case).

Zusammenfassung. Die i.v. Infusion von 1 mg Histamin-2HCl in 30 ml bei einer konstanten Strömung von 1 ml/min während 30 min führt zu einer statistisch signifikanten Abnahme der spontanen kortikalen Delta-Aktivität beim Kaninchen. Dieser elektrographische Weck-Parameter, der gleichzeitig mit einer Desynchronisierung des EEG einhergeht, ist bedeutend grösser bei Kaninchen, die Histamin erhalten haben, als bei Kontrolltieren. Die Weckreaktion dauert nach Ende der Infusion weitere 30 min an. Gleichzeitig bewirkt Histamin ein geringfügiges, nicht signifikantes Absinken des arteriellen Blutdruckes, eine Zunahme der Herzfrequenz sowie ein leichtes Ansteigen der Atmungsfrequenz. Aus diesen Befunden geht hervor, dass Histamin bei unwichtigen visceralen Auswirkungen bei der Regulierung des Wach-Schlaf-Zustandes eine Rolle spielen könnte.

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The Hexose Content of the α-Chains of Bone Collagen

Hexoses and substances which react as hexoses have been found in the collagens of a wide variety of tissues[1–4]. However, it has not been possible to analyze the highly purified single-chain α-components of collagen, because of the leaching out of anthrone-reacting substances from the CM-cellulose resin used to chromatographically separate the α-chains. The recent development of methods for the separation of the 3 α-chains of bone collagen by filtration through Bio-gel P-300 resin (Bio-Rad Laboratories, Richmond, California, USA) and free flow electrophoresis[5] has provided us with an opportunity to analyze the purified single α-chains of a collagen, which have not been subjected to CM-cellulose chromatography.

Bone gelatin was prepared from powdered 12–14-week-old chicken metatarsal bones by methods previously described[6]. The third and fourth LiCl and KSCN extracts were used since these were found to contain no detectable

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