

Influence of Ketamine on the Spontaneous Motility of Chick Embryos and Its Development

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Summary

The effects of acute and chronic application of ketamine on the resting spontaneous motility, its development and reactivity was studied in chick embryos of white Leghorns. 1. Acute application of ketamine (Narcamon[®]) in a dose of 12.5 mg/kg e.w. partially depressed spontaneous motility as early as in 11-day old chick embryos. From day 15 of incubation ketamine very effectively blocked spontaneous motility. 2. Ketamine was fully ineffective in spinal preparations (decapitation on day 2 of incubation) of 11- and 13-day-old embryos. It was not until day 15 evoked that it depressed motility as in normal embryos. 3. Chronic continuous supply of ketamine (average dose 6.34 ± 0.72 mg/kg e.w./24 h) from day 4 of incubation till day 8, 12, or 16 of incubation reduced the developmental decrease of spontaneous motility by 23.1 – 6.0 % as compared to the controls. This effect was already observed after the first 4 days of chronic application of ketamine. 4. Chronic application of ketamine significantly diminished the strychnine activation and GABA-mediated depression of spontaneous motility. The depressive effect of the acute application of ketamine itself was hardly affected. The results have shown that ketamine interferes with the development of the endogenous rhythm of intrinsic activity and with the development of reactivity of the generator of embryonic spontaneous motility.

Key words

Chick embryo – Embryonic motility – Ketamine – Strychnine – GABA

Introduction

Our first attempt to prove the participation of excitatory amino acids in the development and in the central mechanism of embryonic spontaneous motility had not been too convincing, because, first of all, the systemic administration of both glutamate and aspartate did not result in activation, but in a depression of spontaneous motility. The sensitivity of the CNS in these experiments increased from day 11 of incubation in relation to the embryonic age (Sedláček 1978). This could possibly be explained by the difficulty to differentiate between the metabolic and neurotransmitter pool of these two excitatory amino acids (Headley and Grillner 1990).

The marked effect of kainate on the development of spontaneous motility of chick embryos revived interest in problems concerning the participation of excitatory amino acids in the embryogenesis of motor behaviour in chick embryos (Sedláček and Faltin 1989). In this situation, the use of ketamine appeared to be a very attractive approach for detecting elements sensitive to excitatory amino acids, because of its role as a noncompetitive antagonist of NMDA receptors, acting on Na^+ , K^+ and Ca^{2+}

channels in the postsynaptic membrane. The efficacy of ketamine (similar to phencyclidine and MK 801) is derived from its binding on the inner surface of lumen of ionic channel (Krogsgaard-Larsen 1992). It follows from this fact that the typical inhibitory effect of ketamine may disclose the presence of active NMDA receptors in developing central neuronal systems. Such an effect was confirmed *in vivo* on simple models (Yamamura *et al.* 1990), *in vitro* on neocortical cultures (Weiss *et al.* 1986) and on cortical spreading depression (Hernandez *et al.* 1987, Gorelova *et al.* 1987). A similar effect is evident in behavioural reactions (Dalo and Larson 1990) and their development (Marešová *et al.* 1989). In this study acute and chronic administration of ketamine was employed for testing the sensitivity of central generator of embryonic spontaneous motility and its development in the presence of the NMDA-operated mechanism.

Material and Methods

The experiments were carried out on chick embryos of white Leghorns (from a local hatchery),

incubated after preparatory refrigeration (+4 °C for 3 days) under conventional conditions (38 °C, 60-70 % of relative humidity, forced air circulation, automatic turning of eggs every 3 hours). The spinal preparations were obtained by surgical decapitation of embryos aged 2 days of incubation (stage 11-13 according to Hamburger and Hamilton 1951). The embryo's head was sucked off with a glass micropipette after cutting off at the lower border of the 3rd brain vesicle. The lateral window in the shell was closed by a cover-glass fixed to the shell with a parafin mixture. The operated eggs were returned to the incubator and incubation continued under normal conditions (Sedláček and Doskočil 1978).

The motility of chick embryos was recorded by the vibration technique (Sedláček 1977) making possible longlasting records of embryonic motility in eggs and of intact embryos at the time of registration. The graphic record and the frequency and amplitude analysis on a laboratory computer started after 10 min of rest after transfer of the egg from the incubator into the registration box.

In the case of acute application of the tested drugs a small opening was made 24 h beforehand with a dental drill into the air chamber of the egg for spreading the solution over the paper membrane.

Chronic ketamine application was carried out by continual suction of the tested solution from the intact surface of the paper membrane in the extent of the lateral shell window (5x5 mm). This manner of continual application was described in detail elsewhere (Sedláček 1988). Ketamine was administered in this way in 3 experimental groups: in series A from day 4 to 8 of incubation, in series C from day 4 to day 12, and in series E from day 4 to day 16 of incubation. The registration of spontaneous motility was carried out on day 17 of incubation.

Ketamine (NARCAMON^R) in the acute application was given in a dose 12.5 mg/kg e. w. (50 μ) (Marešová *et al.* 1989, Clifford *et al.* 1990, personal pilot study). During chronic application the average dose was 6.37 ± 0.72 mg/kg e.w./24 h at a suction rate of 380 μ l/24 h. Strychnin (Strychninum nitricum^(R)) was applied in a dose of 1 mg/kg e.w. (50 μ l), GABA in a dose of 100 mg/kg e.w. (50 μ l). In acute experiments metrazol was applied in a dose of 100 mg/kg e.w. (Sedláček 1987) and chlordiazepoxide 5 mg/kg e.w. (Sedláček 1989). Resting motility was registered 60 min and in acute testing 20 min before application of the testing solution and 60 min after.

The results of individual groups of animals comprising at least 10 embryos were evaluated by Student's-test using results of a group of minimum 10 embryos.

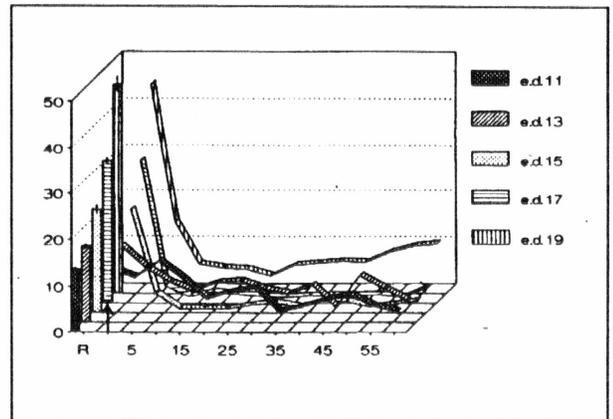


Fig. 1

The effect of acute application of ketamine (12.5 mg/kg e.w.) on spontaneous motility in normal chick embryos. *Abscissa*: R- resting motility during 20 min before ketamine application (arrow); time in minutes after ketamine application. *Ordinate*: frequency of spontaneous movements per minute ($M \pm S.E.M.$). Axis z: embryonic age in days of incubation.

Results

The development of the ketamine effect on spontaneous motility in chick embryos after acute application is summarized in Fig. 1. Even in 11-day-old embryos there was a statistically significant depression of spontaneous motility, which developed in the consequent 60 min. Its onset in 11- and 13-day-old embryos was gradual and required 15 to 20 minutes to reach significant values. At this age, ketamine depressed motility to 59.1 % and 45.2 % of the resting frequency of embryonic movements respectively.

An essential change was observed in 15-day-old chick embryos: the depression of motility developed more rapidly and already reached a maximum during the first 10 min. The remainder of spontaneous motility amounted on the average to 5.9 % (16.6–1.0) of resting value before ketamine application. The same result was recorded in 17-day-old chick embryos: the frequency of spontaneous movements decreased to 4.5 % (10.3–2.1) of resting motility. A somewhat smaller reduction was found in 19-day-old embryos: the spontaneous motility was depressed to 15.6 % (32.6–6.9) of resting motility on the average.

In spinal preparations, where the spinal apparatus developed from day 2 of incubation without any supraspinal influences the motility after acute ketamine application did not change at first. This was the case in 11 to 13-day-old preparations. Only in 15-day-old spinal preparations the motility decreased to 10.1 % (39.7–1.2) after ketamine application on the

average, and to 15.5 % (46.5–5.5) of resting activity in 17-day embryos (Fig. 2)

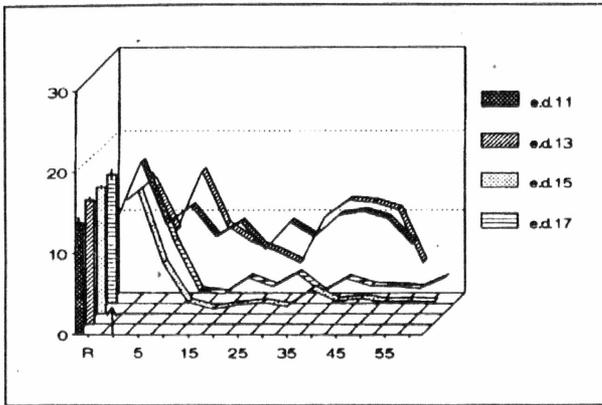


Fig. 2
The effect of ketamine on spontaneous motility in chronic spinal preparations of chick embryos. (Description as in Fig. 1).

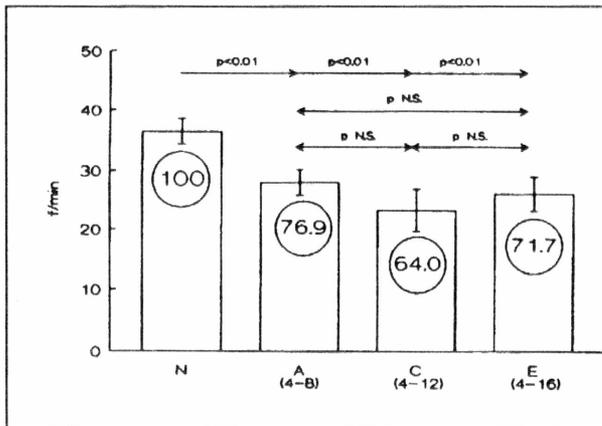


Fig. 3
The consequences of chronic ketamine application on the development of spontaneous motility in normal chick embryos. *Abscissa* - experimental groups: N – normal embryos; A,C,E: experimental embryos with different duration of ketamine application (noted in days in parentheses). *Ordinate*: frequency of spontaneous movements ($M \pm S.E.M.$) in 17-day-old chick embryos. The percentage values of motility in normal embryos are encircled ($N = 100\%$). The horizontal bars give the statistical significance of differences between the experimental groups of embryos.

The study of the effects of chronic administration of ketamine on the development of central motility apparatus from day 4 of incubation, gave the following results. The resting motility of 17-day-old embryos (evaluated as the mean frequency of

spontaneous movements during 60 min of registration) was diminished in all three series with a different duration of chronic ketamine supply (Fig. 3.). The depression of motility was significant in all three series as compared with control embryos. The differences between the experimental series were not significant: motility was reduced by 25 % and this reduction was significant even in series A where ketamine was supplied from day 4 to day 8 of incubation only.

In certain cases the reactivity of the central motor apparatus. was altered. The changes were evaluated at 5/min intervals, at which the evoked change attained maximum values. The strychnine activation of spontaneous motility of 17-day-old embryos was significantly diminished (Fig. 4). A maximum decrease of strychnine activation was found in series A. This unfavourable effect of chronic application of ketamine diminished with the duration of ketamine supply.

In the same way, the strychnine-induced activation was lowered and the GABA-mediated inhibition was also affected (Fig. 5). In this case, the absence of GABA inhibitory effect was most apparent in series E, in which the development was influenced continuously by ketamine between day 4 and 16 of incubation. The both these tests are important, because the development of the central motor apparatus under the continuous influence of ketamine significantly changed the reactivity to acute application of ketamine (12.5 mg/kg e.w.) alone.

Table 1

Maximum depression of spontaneous motility in 17-day-old chick embryos evoked by acute application of ketamine (12.5 mg/kg e.w.) after continual ketamine pretreatment of different duration

Experimental group	n	Resting motility (f/min)	Maximum depression (f/min)
Controls	10	36.4 ± 2.10 (100 %)	0.8 ± 0.50 (2.2 %)
Series A	10	29.1 ± 2.18 (100 %)	1.5 ± 0.39 (5.1 %)
Series C	10	23.5 ± 2.57 (100 %)	1.3 ± 0.35 (5.5 %)
Series E	10	26.1 ± 1.83 (100 %)	1.6 ± 0.54 (6.1 %)

While in normal embryos ketamine depressed resting activity to 4.53 % on the average the depression in

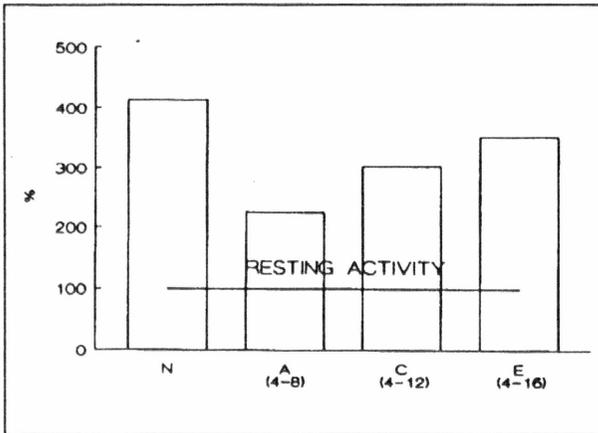


Fig. 4

Maximum strychnine activation of spontaneous motility in 17-day-old normal embryos (N) and after ketamine pretreatment of different duration (A,C,E). Strychnine activation is expressed in percentage of resting motility (= 100 %) before strychnine administration (1 mg/kg e.w.). (Description of the abscissa is the same as in Fig. 3).

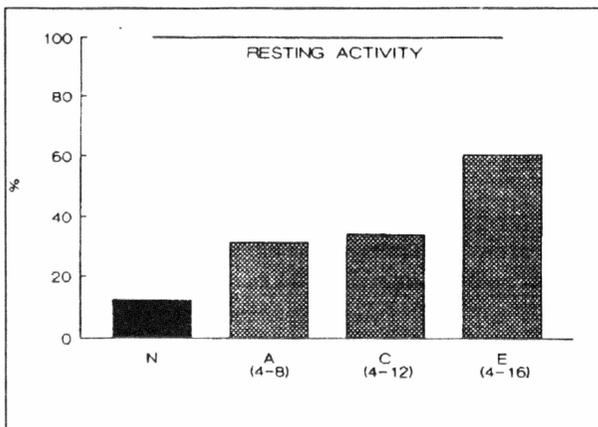


Fig. 5

Maximum depression of spontaneous motility after acute application of GABA (100 mg/kg e.w.) in embryos after different periods of continual pretreatment with ketamine. The frequency of spontaneous movements is expressed in percentage of resting motor activity before the acute application of GABA. (Description of spontaneous motility is the same as in Fig. 3).

series A was decreased to only 15.4 % ($p < 0.001$) and in series E to only 16.2 % ($p < 0.05$) of the resting activity before acute application of ketamine (Tab. 1).

Along with these positive proofs of the consequences of continuous influence of ketamine on

the motor development in some cases no changes of reactivity of neurones in the central motility generator were noted. For example ketamine failed to affect the metrazol paroxysmal activation (metrazol in a dosis 100 mg/kg e.w.) (Sedláček 1987) or the depressive effect of chlordiazepoxide (5mg/kg e.w.) (Sedláček 1989).

Discussion

If we assume that in chick embryos the mechanism of the ketamine effect is basically the same as in adult brain tissue (Yamamura *et al.* 1990, Vyklický 1990), ketamine finds target elements for its effect in 11-day embryos, i.e. ionic channels of NMDA-receptors are present in the neuronal membranes (Lodge and Collingridge 1990). The change noted after day 15 of incubation is quantitative in character, i.e. an enhancement of ketamine effect. The systemic administration, without any doubt, complicates a simple interpretation of the basic findings, since ketamine could also exert an unspecific effect. But this possibility cannot be avoided in our experimental arrangement.

An important finding seems to be the insensitivity of the chronically decentralized spinal cord to ketamine in 11- and 13-day-old spinal embryos and lower sensitivity in 15- and 17-day old preparations. It is possible to speculate that complete and longlasting removal of all supraspinal influences upon spinal neuronal development will also be manifested in impaired development of the receptor endowment of neuronal membranes. The principle of "down regulation" also can not be excluded. This could be the result of the diminished efficacy of the acute application of ketamine in embryos after chronic pretreatment of developing spinal neurones. The maximal effects obtained in series A, although this concerned the shortest exposition to ketamine, attest to a greater interference in neuronal development, namely during the early period of embryogenesis. These changes are evidently due to developmental deviations similarly as the absence of strychnine activation, weakening of GABA-ergic inhibition or the changed reactivity of the ketamine-sensitive component of the NMDA-receptor complex (Sedláček 1990).

At the present stage of our research, is not possible to draw any further theoretical conclusions or speculations. Several open question still remain to be elucidate. Firstly, why ketamine does not affect metrazol activation and chlordiazepoxide depression of embryonic spontaneous motility, and secondly, how specific is the developmental effect of chronic systemic administration of ketamine. Further data may be expected from the study of direct NMDA effects evoked in the neuronal terrain influenced by ketamine pretreatment during development.

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Reprint Requests

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