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Interaction Between Glycine and Glutamate in the Development of Spontaneous Motility in Chick Embryos

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Summary

In this study we investigated whether also glycine fullfils the function as co-activator in glutamatergic activation of NMDA receptors in the neuronal apparatus of spontaneous motility in chick embryos.The successive application of glycine (5 or 10 mg/kg egg weight (e.w.) and glutamate (15 mg/kg e.w.) in a 10 min interval significantly increased the activation of spontaneous motility of 17-day-old chick embryos in comparison with the effect of glutamate alone. This effect did not depend on the order of application of the drugs. In 13-day-old embryos, glycine was ineffective in both doses. It is concluded from these results that the modulatory effect of glycine is evidently a later developmental acquisition (after day 15 of incubation) in the embryogenesis of NMDA-ergic activation of spontaneous motility in chick embryos similarly as glycinergic inhibition.

Key words

Chick embryo – Spontaneous motility – Glycine – Glutamate – NMDA receptor

Introduction

This study was stimulated by interesting experimental findings on some presumed functions of NMDA receptors and glutamatergic synaptic transmission, and especially on the cooperation of glutamate and glycine in activating the NMDA receptor (Johnson and Ascher 1987, Forsythe *et al.* 1988, Kleckner and Dingledine 1988, Lester *et al.* 1993). Kemp and Leeson (1993) stated that the NMDA receptor requires for its activation the occupation of two binding sites, one with glutamate and the other with glycine. This fact makes this type of receptor quite exceptional. It means that the signal molecule of glycine has two fully antagonistic roles: inhibitory neurotransmitter and co-agonist in glutamatergic activation of NMDA receptors (Thomson 1989). The second role requires very low concentrations of this inhibitory amino acid. The same presumption may be derived from findings of Laube *et al.* (1993).

The idea that glycine modulates the glutamate effect on NMDA receptors is very attractive from the point of view of the embryonic development of spontaneous motility and its neuronal basis (Sedláček 1978, 1982). Very favourable was the fact that the inhibitory effect of glycine on spontaneous motility begins to manifest itself approximately from day 15 of incubation (Sedláček 1977a). For that reason the age of embryos in this study was chosen so as to cover the period before and after this important landmark of embryonic development.

Material and Methods

The experiments were carried out on chick embryos of white Leghorns (average weight around 50 g) aged 13 and 17 days of incubation according to the above-mentioned consideration based on much experimental evidence (Sedláček 1987). Fertile eggs (from a local hatchery) developed under usual conditions of temperature, air humidity, automatic egg turning and forced air exchange in a laboratory incubator.

Glycine and glutamate were acutely applied by spreading 50 μl of the tested solution on a paper membrane with fine polyethylene tubing introduced through a tight opening in the shell into the air chamber of the egg. Na^+-L-glutamate (Sigma) was administered in a dose of 15 mg/kg egg weight (e.w.), glycine (Sigma) in doses of 5, 10 and 100 mg/kg e.w. (both amino acide in 50 μl of physiological saline).

Spontaneous motility of chick embryos was recorded from intact incubated eggs using the vibration technique (Sedláček 1977b) connected with a graphic recorder. Frequency and amplitude analysis of spontaneous movements was made by the computer.

The experiments were arranged as follows: first the resting motility was registered for 20 min, then the tested solution was applied and the registration continued without interruption for the next 60 min. In the experiments with interaction of the tested drugs, the amino acids were applied at intervals of 10 min; the motility registration continued after the last administration for another 60 min.

In each experimental group, the results (M ± S.E.M.) were evaluated in 10 embryos of appropriate incubational age. Student's t-test was used for statistical evaluation of the results.

Results

The first experiments carried out on 17-day-old chick embryos were to answer the basic question whether a relationship between the effect of both amino acids detectable in this pattern of embryonic spontaneous motility is present at all in chick embryos at this age. The results of three experimental series were compared: in the first series glycine was applied in a dose 100 mg/kg e.w., in the second series Na^+-L-glutamate alone was given in a dose 15 mg/kg e.w. and in the third decisive series Na^+-L-glutamate was applied in a 10-min interval following glycine application (10 mg/kg e.w.).

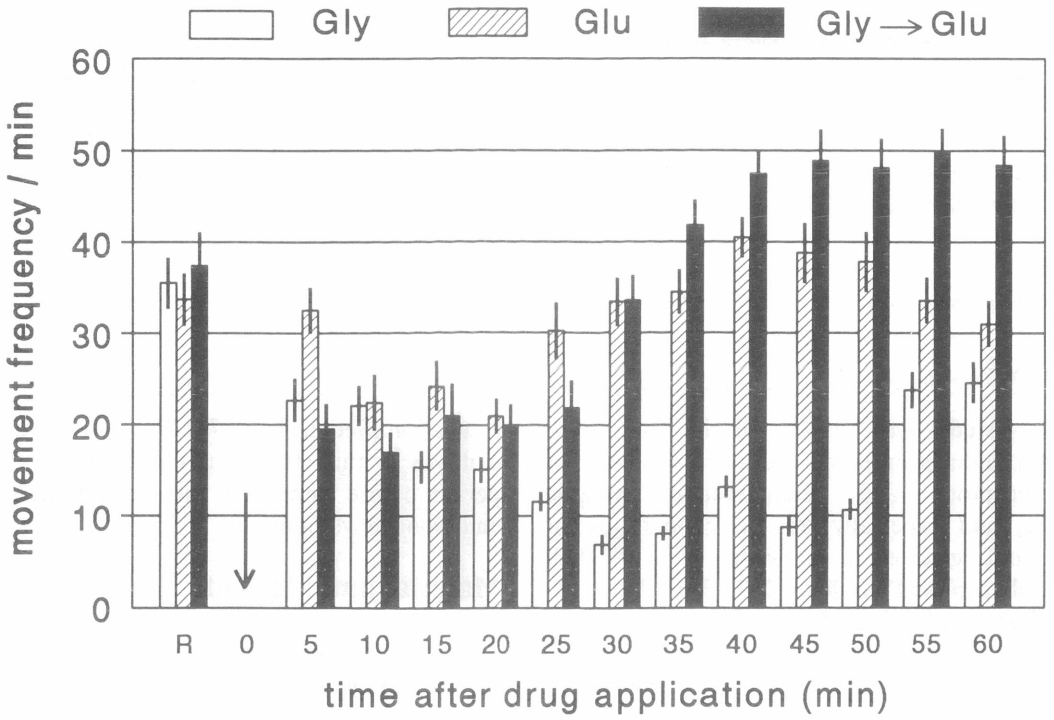

Fig. 1
The spontaneous motility changes in 17-day-old chick embryos after the separate application of glycine (Gly, 100 mg/kg e.w., open columns), Na^+-L-glutamate (Glu, 15 mg/kg e.w., hatched columns) and after the successive application of glycine and glutamate (Gly- - >Glu, 10 and 15 mg/kg e.w., full columns). R – resting activity during 20 min before application (arrow) of amino acids.

It turned out that (Fig. 1), glycine alone significantly reduced spontaneous motility to 20 % of the resting value (Sedláček 1982) as proof of sensitivity to this inhibitory amino acid at this embryonic age. Glutamate alone manifested itself after a short initial depression (Sedláček 1978) by only a moderate and insignificant activation of resting activity by 12–20 %. Contrary to this, however, following the successive application of both amino acids in the order glycine-glutamate, a marked and highly conclusive increase of activatory effect of Na^+-L-glutamate developed within the second half of experiments. The motility of 17-day-old embryos increased by 27–33 % in comparison with the resting activity ($p<0.05$ to $p<0.001$ for values at 5-min intervals).

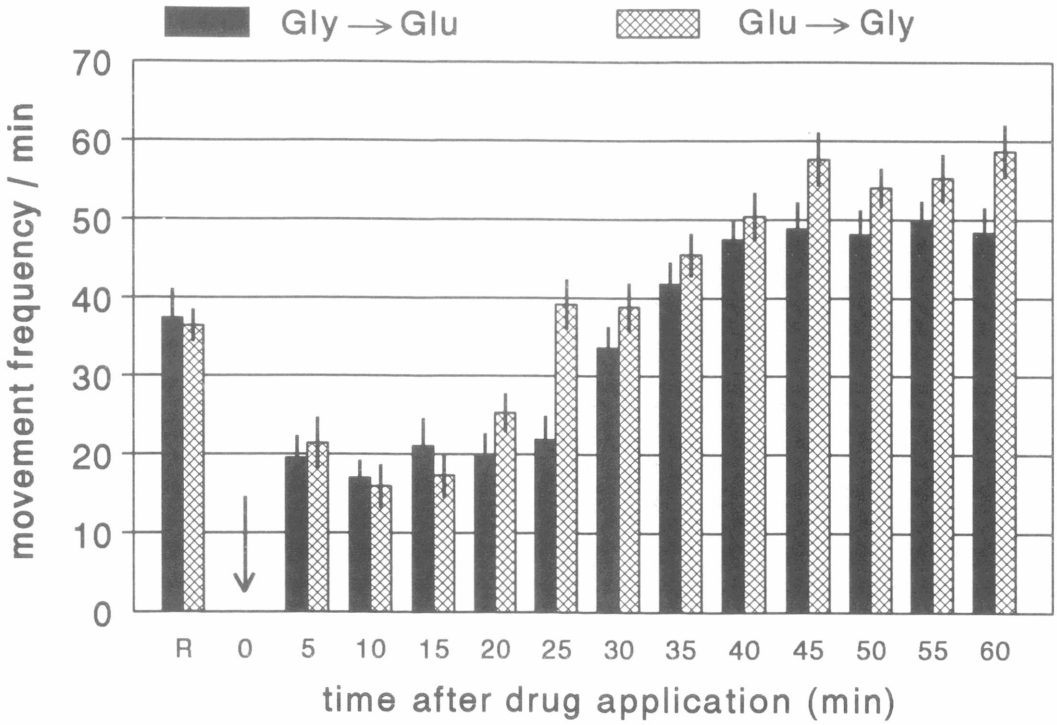

Fig. 2
The spontaneous motility changes in 17-day-old chick embryos following the successive application of glycine and glutamate (Gly- - >Glu; full columns) and glutamate and glycine (Glu- - >Gly; cross-hatched columns). For other details see Fig. 1.

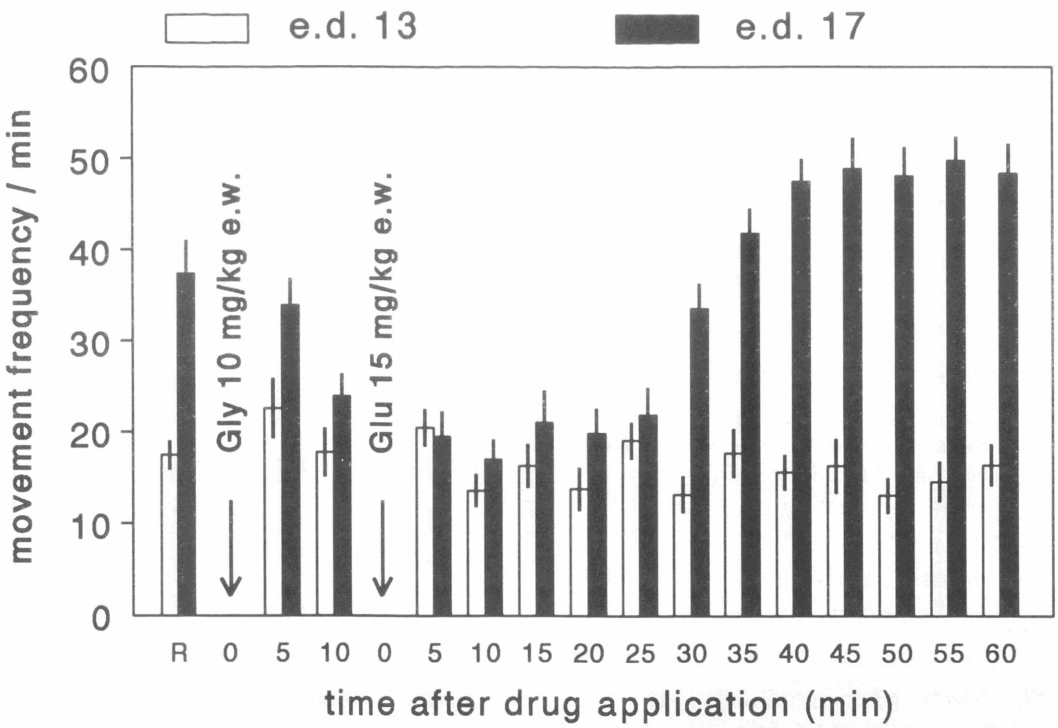

Fig. 3
The spontaneous motility changes in 13-day-old (open columns) and 17-day-old chick embryos (full columns) following successive application of glycine (Gly; 10 mg/kg e.w.) and glutamate (Glu; 15 mg/kg e.w.). For other details see Fig. 1.

A similar result was obtained after the application of both amino acids in similar doses, but in a reverse order (Fig. 2). Likewise, in such an arrangement, the increase of glutamate activatory effect occurred during the second half of the experiment: in this case the increase of motility exceeded 50 % of resting activity ($p<0.02$ to $p<0.01$) during the last four 5-min intervals. The results in both groups with a different sequential application of the two amino acids were not significantly different.

With respect to the positive results of the first group of experiments, it was possible to investigate the positive effect of glycine and the possibility of its dose-dependence (Kemp and Leeson 1993). The experiment carried out on 13-day-old embryos in a similar manner showed that glycine (Fig. 3) in the dose 10 mg/kg e.w. was fully ineffective at this embryonic age. It neither depressed motility (within the first 10 min after glycine application in 17-day-old embryos) nor activated it during 60 min after the addition of glutamate. The spontaneous motility of 13-day-old embryos did not change significantly during the whole experiment. The same result was obtained after the application of half the dose of glycine: 5 mg/kg e.w. (Fig. 4). This strategy was chosen because of 1. the demand of very low glycine concentrations sufficient to saturate the glycine site (Kemp and Leeson 1993), 2. the different sensitivity of developing embryonic brain tissue, and 3. the ineffectiveness of 100 mg/kg e.w. of glycine in 13-day-old chick embryos (Sedláček 1977a). Whilst the results in 13-day-old embryos were unambiguously negative, in 17-day-old embryos the lower dose of glycine did not ensure such marked and stable glutamate activation of motility (Fig. 4) as the double dose (Fig. 3).

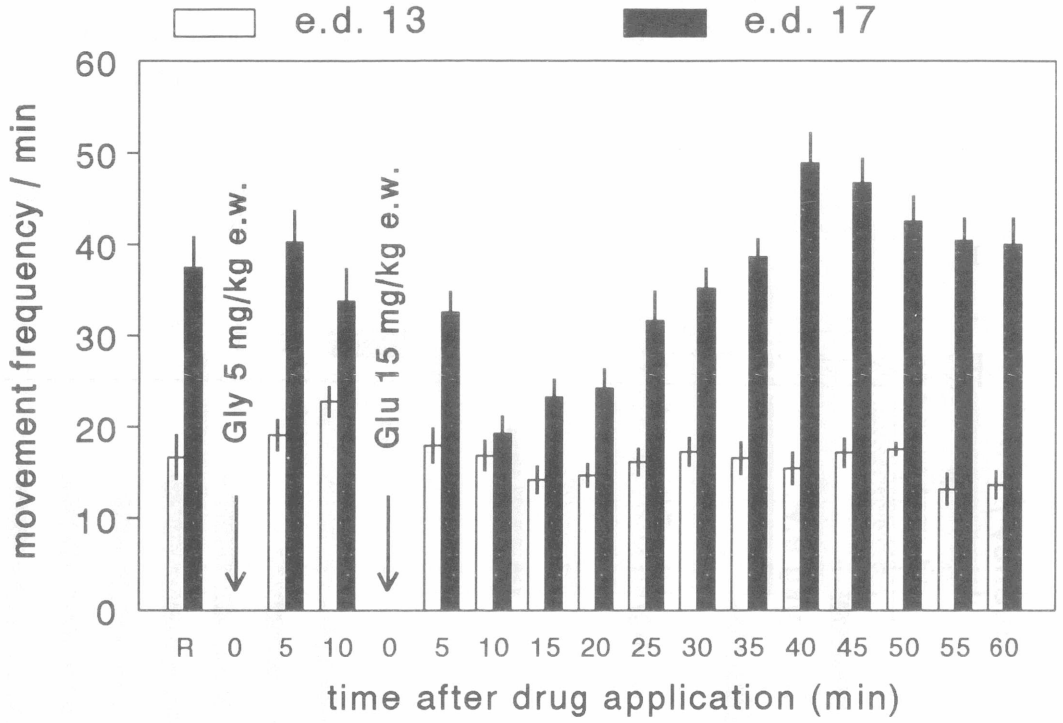

Fig. 4
The spontaneous motility changes in 13-day-old (open columns) and 17-day-old chick embryos (full columns) following successive application of glycine (Gly; 5 mg/kg e.w.) and glutamate (Glu; 15 mg/kg e.w.). For other details see Fig. 1.

Discussion

This study was not aimed to analyse the molecular phenomenon of glycine modulation of glutamate-operated activation but to detect this phenomenon in such a behavioural pattern as the developing spontaneous motility in chick embryos.

The results with the successive application of both amino acids in 17-day-old chick embryos, i.e. the glycine potentiation of the activatory effect of glutamate, are not in conflict with the idea of the glycine role in the modulation of glutamatergic transmission *via* NMDA-receptors. Remarkable is the finding that glycine was without any effect in 13-day-old chick embryos as both the inhibitory neurotransmitter

and the co-activator of the NMDA-mechanism. In the first case, the glycine ineffectiveness prior to day 15 of incubation is in agreement with the course of embryonic development of central inhibition (not only the glycine but also of the GABA-ergic type) (Sedláček 1982). In the second case, there is a controversy with the activatory effect of NMDA and with the depressive effect of ketamine even before day 15 of incubation (Sedláček 1992). The effects of both drugs are mediated by the same NMDA receptors.

The basic question may be put in two different ways: either glycine is ineffective alone before day 15 of incubation as the neurotransmitter and the neuromodulator, or the modulatory glycinergic component is later acquired in the course of development of embryonic NMDA-ergic mechanism of spontaneous motility. The second possibility could weaken the rigorous standpoint on the role of glycine as "the absolute requirement" for successful activation of the NMDA receptor (Kemp and Leeson 1993). Nevertheless, the possible participation of the development of glutamate-ergic mechanism and its influence on the glycine effect during the process of activation of the NMDA receptor with a specific ligand (Ragozzino and Eusebi 1993) cannot be excluded from this consideration.

However, the present results have to be considered from another point of view. Sedláček (1978) reported some insignificant influence of glutamate on the spontaneous motility in chick embryos. This could be explained by the dose of glutamate: the dose in those experiments was threefold higher than the present dose and for this reason the paradoxical effect cannot be excluded. From the point of view of the present findings it would be possible to look for the explanation in the parallel development of glutamatergic and glycinergic component and in an insufficient positive modulatory influence of natural concentrations of glycine in the developing embryonic brain tissue.

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