Physiol. Res. 41: 115-120, 1992

Interaction of Beta-Carboline with Chick Embryo Spontaneous Motility

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Received June 27, 1991
Accepted January 2, 1992

Summary

The effect of beta-carboline (β-CCE) on spontaneous motility and its development was studied in chick embryos between the 11th and 19th day of incubation. 1. Acutely administered β-CCE (7.5 mg/kg e.w.) already induced significant activation of motility in 11-day-old embryos. From the 17th day of incubation activation acquired a paroxysmal character. 2. In spinal embryos (decapitated on the second day of incubation) there was no such activating effect, demonstrating that it is associated with supraspinal components of the CNS. 3. In chronic administration from the fourth day of incubation (1.55 ± 0.24 mg/kg e.w./24 h), β-CCE led to reduced development of spontaneous motility. The effect was concentrated in the period between the fourth and eighth day of incubation. The chronic administration of β-CCE augmented the activating effect of metrazol and weakened GABA-inhibition of spontaneous motility. 4. On the basis of their findings, the authors express the hypothesis that the benzodiazepine β-CCE-sensitive component of the complex GABA receptor evidently already functions from the beginning of the second half of incubation of chick embryos.

Key words

Chick embryo – Embryonic motility – β-carbolines – CNS – GABA receptor

Introduction

The attractive problems of functional embryogenesis of the CNS indubitably include the problem of the development of central inhibition as one the basic regulatory elements. It has been estimated that GABA is the neurotransmitter on about 30 % of the central synapses in the adult brain. Elimination of this process leads to disinhibition of postsynaptic neurones (Prince and Gutnick 1972, Somjen *et al.* 1978). The first signs of convulsive activity are therefore a valuable indication that complex GABA receptors are beginning to function (Sedláček 1982, 1983a).

While bicuculline identifies the GABA-ergic component and picrotoxin the chloride ionophores of the complex receptor, β-carboline is a means of identifying the benzodiazepine component of this receptor. Tenen and Hirsch (1980) and Cowen *et al.* (1981) showed that the acute administration of β-carboline had a proconvulsant effect, i.e. that it potentiated the activity of convulsants like metrazol or bicuculline. Furthermore, Biggio *et al.* (1989) found that the long-term administration of benzodiazepines and β-carbolines had a similar effect, i.e. down-regulation of the $GABA_A$ (benzodiazepine) ionophore receptor complex, and supplemented this information by behavioural and biochemical findings showing that the function of the $GABA_A$ receptor complex can be depressed by the repeated adminstration of β-carbolines (Biggio *et al.* 1989). Lastly, Braestrup *et al.* (1980), Saano and Airaksinen (1982) and Costa *et al.* (1983) did not exclude the possibility that substances of the β-carboline group might be natural ligands of benzodiazepine receptors modulating the function of the whole GABA receptor.

The above information in the literature furnishes sufficient grounds for using β-carbolines to study the role of the central GABA-ergic mechanism in the development of embryonic spontaneous motility (Sedláček 1987).

The aim of the present study was to determine whether actually administered β-carbolines are capable of inducing paroxysmal activation of spontaneous motility in chick embryos and what are the developmental effects they have when administered chronically.

Material and Methods

The experiments were carried out on White Leghorn embryos (from a local farm) incubated under usual standard conditions (temperature, humidity, turning the eggs over, etc.). Development was synchronized by refrigerator preparation of the eggs (Gottlieb 1963)

Spinal embryos were obtained by surgical decapitation at the end of the second day of incubation, at stage 11–13 (Sedláček and Doskočil 1978). Spontaneous motility of the embryos was recorded by the vibration technique in intact incubated eggs (Sedláček 1977).

In acute administration experiments the following test solutions were sprayed through a small opening in the shell over an area of the egg paper membrane corresponding to the extent of the air chamber: ethyl-β-carboline-3-carboxylate (β-CCE) (RBI, Wayland, USA) in a single dose of 7.5 mg/kg e.w. (50 μl); metrazol (SPOFA, Czechoslovakia) in a dose of 100 mg/kg e.w. (50 μl); GABA (SERVA Germany, USA) in a dose of 100 mg/kg e.w. (50 μl).

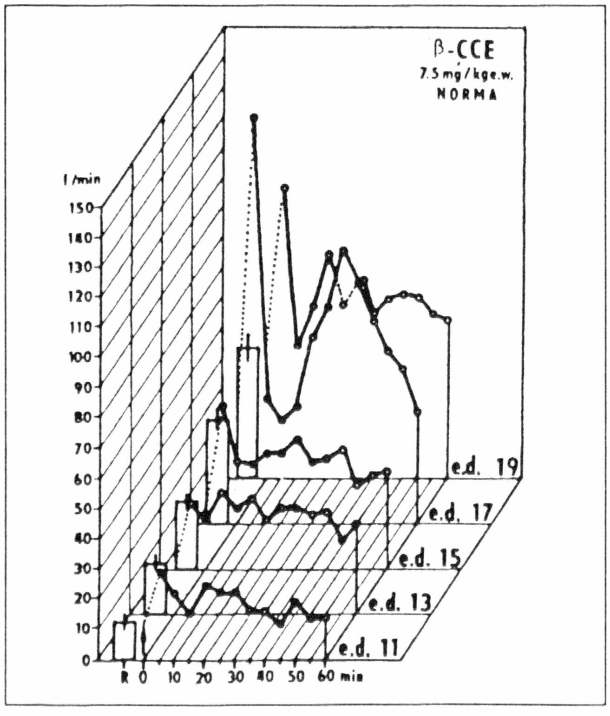

Fig. 1
Development of the effect of b-CCE on spontaneous movement frequency in chick embryos. x axis: R – 20 min resting activity before administering bβ-CCE (M + S.E.M.); time in min after administration (arrow). y axis: spontaneous movement frequency per min. z axis: embryonal age (days of incubation).

In chronic administration experiments, the mean dose of β-CCE was 1.55 ± 0.24 mg/kg e.w./24 h. The solution was administered continuously from a container (Sedláček 1988) from the 4th to the 8th day of incubation (group A), from the 4th to the 12th day (group C) and from the 4th to the 16th day (group E). In this last group the minimum interval between terminating administration and beginning to record motility was at least 24 hours. In these experiments, the embryos' motor activity was recorded at an incubation age of 17 days. All the data in this study are based on the results from at least 10 chick embryos.

Results

The acute administration of β-CCE (7.5 mg/kg e.w.) first evoked brief simple activation of motility in 11-day-old embryos (Fig. 1) – an effect which remained unchanged up to the 15th day of incubation. From the 17th day, however, a single dose produced not only marked quantitative changes, but also changes in the general picture of spontaneous motility, including a prolonged paroxysmal response (Fig. 2) which was generally recorded for a full 60 min after the dose of β-CCE. Another aspect of this development was the striking dependence of the effect of β-CCE on the supraspinal compartments of the brain; a substantial part of the activating effect of β-CCE did not appear in chronic spinal specimens (Fig. 3 and 4).

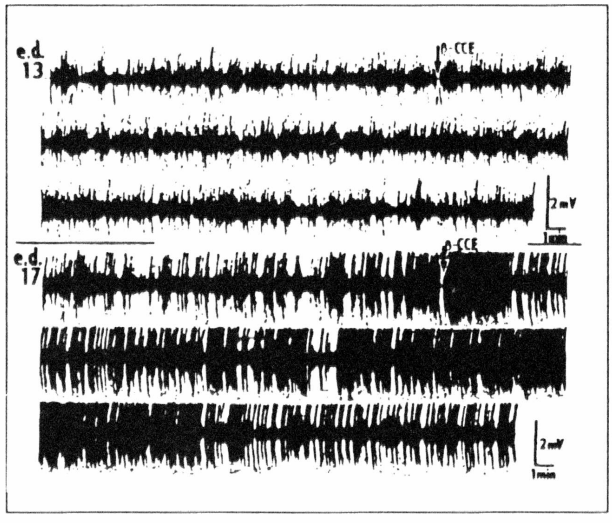

Fig. 2
Original recording of spontaneous motor activity of a 13- and 17-day-old chick embryo after acute administration of b-CCE (arrow). The individual recordings in the upper and lower three traces are continuations of one another. The 2 mV calibration reperesents arbitrary units of the amplitude of spontaneous movements.

The protracted, continuous administrations of β-CCE provided further information on their activity.

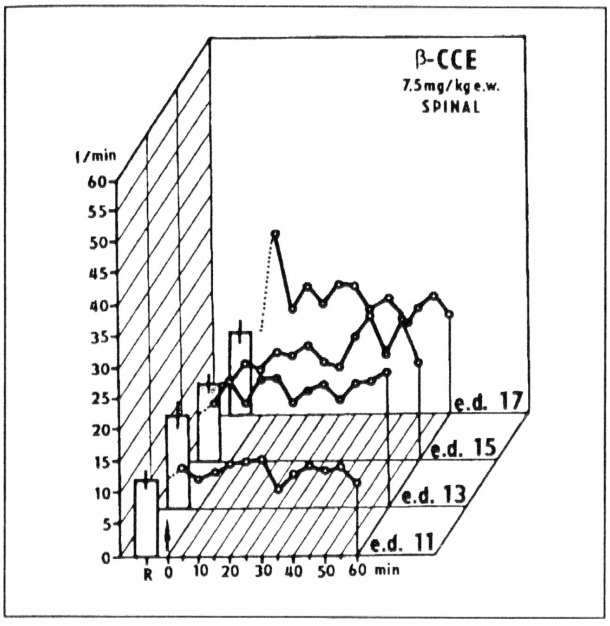

Fig. 3
Effect of β-CCE on the spontaneous motor activity of spinal chick embryos. Details as in Fig. 1.

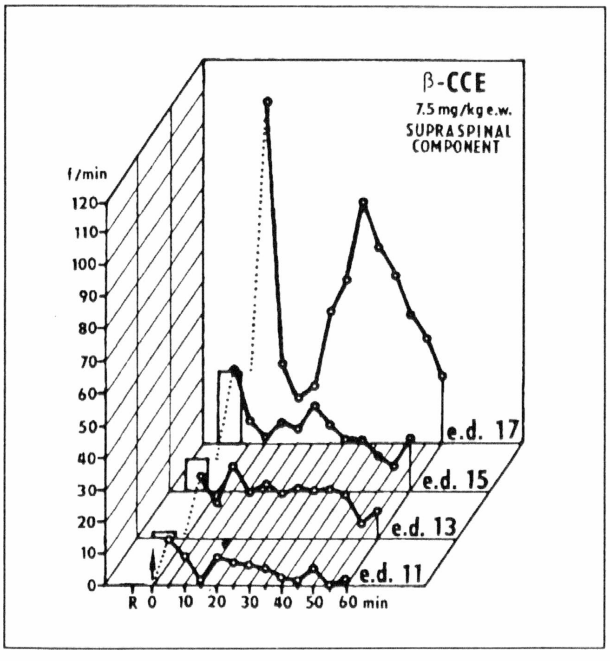

Fig. 4
The supraspinal component of the effect of β-CCE on the spontaneous motor activity of chick embryos. The curves represent the simple differences between the data in Fig. 1 (= total cerebrospinal activity) and Fig. 3 (= the spinal component). Further details as in Fig. 1 and 3.

The main finding was that, in this case, β-CCE significantly reduced spontaneous motility (Fig. 5). The administration of β-CCE from the 4th to the 8th day of incubation only (series A) already gave rise to this developmental defect; prolongation of the administration time (series C and E) did not influence it significantly any further, either positively or negatively. Moreover, no signs of recovery were observed either in series A (9 days before recording motility) or in series C (5 days before). In all three experimental series, metrazol activation was potentiated: in groups C and E more than in group A, so that the prolonged continuous adminstration of β-CCE did have developmental consequences.

It was found in further experiments that it was not only the spontaneous activity of central motor output that was affected by the above developmental effect, but also the development of its reactivity. This was manifested chiefly in the activating effect of metrazol (Fig. 6).

The situation as regards the reaction of spontaneous motility to the acute administration of GABA (Fig. 7) was similar.

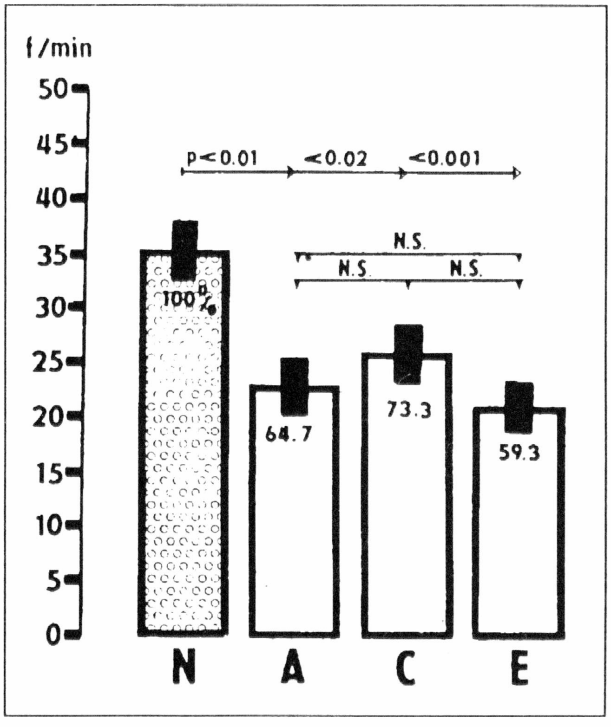

Fig. 5
Results of chronic administration of β-CCE on the development of spontaneous motor activity in 17-day-old chick embryos. *Abscissa*: experimental groups; N – normal control embryos; A,C,E – embryos exposed to β-CCE for different lengths of time (see text). *Ordinate*: spontaneous movement frequency per min (M ± S.E.M.). The percentage of activity in normal embryos (= 100%) is given inside the columns. The horizontal lines show the degree of significance of the differences between the groups denoted by the arrows.

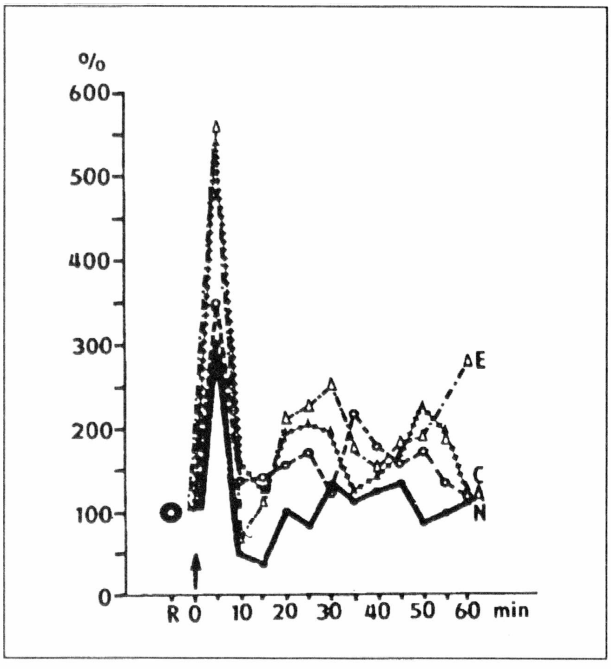

Fig. 6
Metrazol activation of spontaneous motor activity in 17-day-old embryos exposed to β-CCE for different lengths of time. *Abscissa*: resting activity before administering metrazol; time in min after administering metrazol (arrow). *Ordinate*: percentage of resting activity before administering metrazol. N – normal embryos; A, C, E – embryos pretreated with β-CCE for different lengths of time (see text).

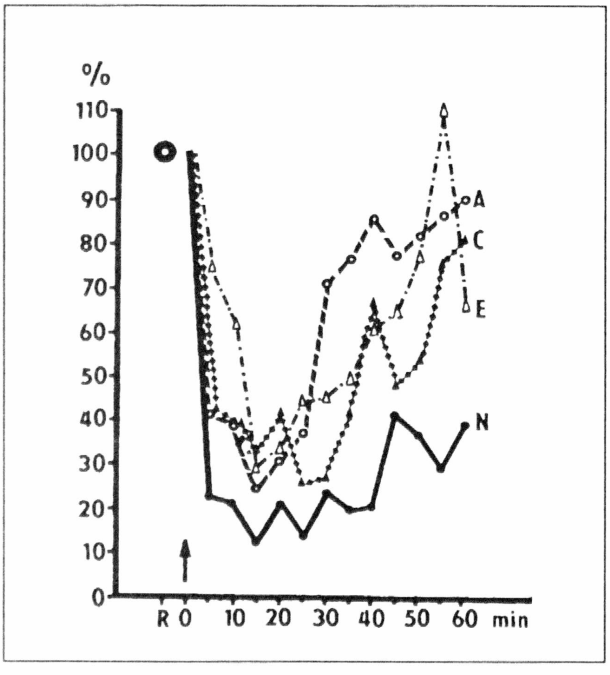

Fig. 7
Changes in GABA-ergic depression of spontaneous motor activity in 17-day-old embryos pretreated with bβ-CCE or different lengths of time. Details as in in Fig. 6.

In general, the inhibitory effect of GABA was attenuated in all three experimental groups, but it was most weakened in group E, in which β-CCE was administered the longest and GABA-ergic inhibition reduced spontaneous motility only to 75 % of resting activity (in the control embryos not exposed to the action of β-CCE it was reduced to 20 %).

Discussion

The aim of this study was to confirm, by means of β-CCE, the existence of benzodiazepine receptors and their developmental characteristics of the spontaneous motility in chick embryos. Previous attempts at such a demonstration generally turned out satisfactorily (Sedláček 1983a, 1989a,b). If β-CCE are endogenous ligands of the benzodiazepine component of the GABA-ergic receptor (Costa *et al.* 1983), and if they evoke corresponding changes in embryonic motility, there must be sensitive benzodiazepine receptors or units in the embryonic CNS from at least the 11th day of incubation onwards. Further development then reveals the development of the proconvulsant effect of β-CCE.

A further finding stems from the difference in the acute effect of β-CCE in normal and spinal embryos. This comparison – but primarily the vagueness of the effect in spinal embryos – leads to the conclusion that the decisive mass of β-CCE-sensitive elements develops in supraspinal structures of the embryonic CNS. However, two contradictory findings speak against this concept. The effect of both GABA and oxazepam on embryonic spontaneous motility undoubtedly has a spinal component. Secondly, whereas the effect of GABA is significant from the 15th day of incubation (Sedláček 1982) and of oxazepam (Sedláček 1983a) and chlordiazepoxide from the 13th day (Sedláček 1989b), β-CCE is already unquestionably effective in 11-day-old embryos.

This conflict of experimental results can be bridged for the time being by assuming that the embryonic development and functional maturation of the individual components of the complex GABA receptor are heterochronous.

Another serious problem is the time disparity of the effect of acute and chronic β-CCE administration which accompanies this study of the embryonic development of central inhibition from the very beginning. The chronic effect both of GABA and of oxazepam and chlordiazepoxide, and now of β-CCE as well, is concentrated in the five days from the 4th to the 8th day of incubation and prolongation of their administration never results in any further significant difference.

What is the mechanism of sensitivity of the generator of spontaneous motility to ligands of the complex GABA receptor at the outset of development

of the first motor manifestations of the chick embryo? Despite the evident effects of β-CCE, we failed to demonstrate any changes in the binding proteins after the chronic administration of β-CCE (Sedláček *et al.* 1990). If such changes could be demonstrated, it would greatly facilitate the interpretation of the present results.

Lastly, the results submitted here show that the chronic administration of β-CCE is capable of interfering with the functional development of the central generator of embryonic spontaneous motility. The experimental data do not, however, make it clear whether the generator of spontaneous motility itself is impaired, or whether the GABA-ergic regulatory apparatus is damaged at the spinal cord or supraspinal control level.

The problems outlined above arose from these experimental results and must therefore be resolved in the same manner.

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