Eur J Anat, 11 (2): 85-88 (2007)

Effects of gamma-hydroxybutyrate (liquid ecstasy) on the development of the chick cranium

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SUMMARY

Gamma-hydroxybutyrate (GHB), a precursor to gamma aminobutyric acid (GABA), is currently one of the widest used drugs of abuse, with devastating effects. GHB produces a transient rise in the concentration of dopamine in the brain, inducing a stimulating effect. However, GHB can produce hallucinations, delirium, somnolence, hypotonia, confusion, and loss of balance. There may be subsequent progression to coma, with severe respiratory alterations due to CNS depression. The effects of GHB on humans are unpredictable, and its impact on the embryo and fetus is completely unknown. The purpose of our study was to contribute experimental data regarding the effects of GHB on development.

Groups of chick embryos received 100 µl of GHB at dilutions of either 20% (GHB-A) or 30% (GHB-B) at 7 or 11 days of incubation, a further group serving as controls. After hatching, different cranial measurements were made using a Mitutoyo 500-331 series slide gauge in order to investigate possible effects of the drug on cranial development.

At both doses studied, GHB significantly altered the transverse and vertical cranial measurements in comparison with vehicle-treated controls, suggesting a possible harmful effect on cranial development.

Key words: Chick embryo – Cranial development – Gamma-hydroxybutyrate – Liquid ecstasy

INTRODUCTION

Gamma-hydroxybutyrate (GHB) can be found in all cells of the organism, although in some areas it is more abundant than in others. For instance, in the brain there is a greater concentration in the hypothalamus and basal ganglia. GHB was discovered during investigations into the effects of the neurotransmitter gamma aminobutyric acid (GABA). It was found that GHB, a precursor in its metabolism obtained by replacement of the amino group by a hydroxyl group, was able to traverse the blood-brain barrier, unlike GABA itself. GHB has many features in common with GABA, although there is some debate as to whether it is an agonist (Feigenbaum and Howard, 1996).

GHB was found to have wider effects than those attributed to GABA. It proved to be a potent central nervous system depressant, increasing the concentration of dopamine in the brain (Dzoljic et al., 1975) and leading to a strong feeling of well-being, with an aphrodisiac effects, and an increased state of alertness (Engelsen and Christensen, 1991; Strange and Jensen, 1999).

Submitted: January 31, 2007
Accepted: May 4, 2007

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Before the nineteen-nineties, many benefits of GHB were reported. At the appropriate dose, it could be used as an anaesthetic, is therapy for narcolepsy and even drug dependence (Poldrugo and Addolorato, 1999), and in the treatment of patients with brain lesions (Maslov et al., 1987; Volpi et al., 2000). However, it began to be reported that patients receiving GHB anesthesia suffered from hallucinations and states of confusion. In 1991, the first cases of intoxication were published, revealing its potential as a drug of abuse, and it was given the name of "liquid ecstasy".

Our understanding of its harmful effects has continued to increase. It is now known that GHB is able to produce somnolence, hypotonia, cephalalgia, confusion, and loss of balance. There may be hallucinations, delirium, euphoria, or anesthetic states, with respiratory alterations secondary to CNS depression, and even progression to coma (Ingels, et al., 2000). In many cases, the effects are very fast and intense. (Miotto et al., 2001; Nini et al., 2001; Feldman and Croquett-Krokar, 2002).

A further danger of this drug lies in the impossibility of measuring a precise dose of the liquid in the setting in which it is habitually consumed. Furthermore, because it is odorless, colorless, slightly salty, and water-soluble, it can be introduced into a drink and ingested without the drinker's knowledge. When taken with alcohol or other drugs it can have even more harmful consequences (Degenhardt et al., 2002). Similar to the case of alcohol, the effects are unpredictable, because each individual reacts differently.

As it is a novel drug in relatively recent use, we have found no data on its long-term action or effects on development. The purpose of the present work was to contribute experimental data on the effects of GHB during the embryonic period. This issue is of special interest because GHB users tend to be of fertile age.

Because GHB is a precursor to GABA, its action on the CNS largely occurs when this tissue is being formed. The most active period of neurogenesis is between days 7 and 11 of incubation, when the thyroids, a critical factor in this histogenesis, are functioning.

Here we performed experiments to study the action of GHB on the developing skull, so closely related to the development of the CNS. For this purpose, we reviewed the embryologic calendar of the skull (Romanoff, 1960). We focused on the fundamental elements of cranial development, such as the acrochordal and parachordal cartilages and the otic and nasal capsules. The key dates of their development are between days 7 and 11 of incubation.

The cranium of birds is formed by interrelated bones. Two parts can be distinguished: an upper part, the neurocranium, which contains and protects the brain and sense organs; and a lower part, the viscerocranium, which protects and supports the jaw and tongue. The neurocranium and viscerocranium are composed of bones of membranous origin, which form the vault, and those of cartilaginous origin, which constitute the base on which the brain rests. During its development, the latter comprise a series of independent cartilages that later fuse and ossify, and it is during this period that we believe the action of the drug may be exerted.

The first cartilage to form is at the anterior end, in front of the notochord. This is the acrochordal cartilage, whose posterior end forms the back of the hypophyseal fossa. In the chick, it appears on day 4-5, and its most evident development occurs as from day 6, when it grows transversally, playing an important role in regulating the formation of the cranium in this direction. After the acrochordal cartilage, the parachordal cartilage appears, which grows in the anteroposterior direction and is in full development at day 7 of incubation. Since the time of their appearance, these cartilages are flanked by cochlear portions of the auditory capsule, which in the chick form part of the parachordal cartilage, which grows from day 6.

The auditory capsule is formed by fusion of the canalicular and cochlear portions, and houses the organ of hearing. The two portions eventually fuse, but a posterior space or orifice is left between them for the passage of the statoacoustic nerve. Prior to day 10, this orifice is subdivided by the different branches of the nerve. Behind the otic capsule is the metotic fissure, which is subsequently partly obliterated by the presence of the metotic cartilage, observed at around day 7. The otic capsules on each side connect with these cartilages through the cartilage of the tectum synoticum, which forms the dorsal edge of the occipital foramen and is completed from one side to the other between days 10-11.

The trabeculae cranii appear in the area in front of the notochord. These are two cartilaginous bars in front of the acrochordal cartilage that begin to develop from day 6. By day 7, these trabeculae have fused at their anterior end, forming the common trabecula. From this derive the nasal and interorbital septa and the roof of the nasal capsule. At around day 8

the lateral wall of the nasal capsule is formed. Subsequently, at around day 10 or 11, the cartilaginous roof of the nasal fossa is formed.

Material and Methods

We incubated 168 fertilized Leghorn HR7 eggs, weighing 55-65 g, at 37.8 ± 0.4ºC at a relative humidity of 60-70% in a Masalles Model 65 incubator equipped with forced ventilation and automatic voltage. The eggs were divided equally between four groups: one was injected only with the vehicle (100 µl distilled water); another was injected with dose A (20 µl GHB + 80 µl distilled water); and a third group was injected with dose B (30 µl GHB + 70 µl distilled water). The eggs were injected at 7 and 11 days of incubation, key dates in the development of the cranium, as commented above (Romanoff, 1960). The fourth group was left untouched as a control. Sterile distilled water was selected as the ideal vehicle, because we previously determined that it was harmless (Table 1). The injection was always made into the air chamber, making two 1-mm holes at the larger end of the egg that crossed the cuticle, the shell, and the outer membrane. One hole served to administer the dose and the other to enhance the penetration of the solution. After the injection, both holes were closed with liquid paraffin and the egg was returned to the incubator.

The animals were sacrificed on day 21 of incubation. Fifteen, 15, 19, or 20 eggs were taken from each group, depending on the mortality rate of the group, for the measurement with a Mitutoyo slide gauge of the following representative cranial parameters: transverse diameter (TD), the distance between the two external auditory openings; the anteroposterior diameter (APD), between the root of the crest and the external occipital protuberance; and the vertical diameter (VD), between the right auditory opening and the highest point of the calotte, following the method of Oliver and Pascual (Oliver et al., 1987a, b).

The total body weights and brain weights of the dose- and vehicle-treated chicks were also analyzed (Table 3) in order to exclude any possible relationship between the increase or decrease in these weights and variations in cranial measurements.

The statistical significance of the differences in measurements between the drug- and vehicle-treated groups was established using Student's t test for independent samples. The statistical study was performed with the SPSS Base 10.0 package for Windows.

Table 1. Relationship between cranial parameters (in mm) of embryos injected with water vehicle and controls.

	TD	APD	VD
WV	(N=19) 40.41±5.28	(N=19) 28.02±0.95	(N=19) 12.22±1.10
	n.s.	n.s.	n.s.
C	(N=20) 41.41±4.17	(N=20) 27.91±0.89	(N=20) 12.06±0.92

All values are expressed as means ± SD. N: number in sample; n.s.: not significant; WV: Water vehicle; C: Controls; TD: Transversal diameter; APD: Anteroposterior diameter; VD: Vertical diameter.

Table 2. Effects of GHB-A and GHB-B doses on cranial parameters of chick embryo (mm).

	TD	APD	VD
GHB A.	(N=15) 15.3 ± 0.4	(N=15) 28.1±1	(N=15)13.9± 1.1
	p<0.001	n.s.	P<0.001
WV	(N=19) 14.8 ± 0.4	(N=19) 28.±0.9	(N=19) 12.2±1.1
	p<0.001	n.s.	P<0.001
GHB B.	(N=19) 15.3 ± 0.5	(N=19) 27.5±1.2	(N=19) 13.1± 0.6

All values are expressed as means ± SD. N: number in sample; n.s.: not significant; WV: Water vehicle. C: Controls. GHB A: Treated with GHB A dose; GHB B: tretaed with GHB-B dose; TD: Transversal diameter; APD: Anteroposterior diameter; VD: Vertical diameter.

Table 3. Analysis of total body weight and brain weight of chicks treated with both doses of GHB with respect to those injected with water vehicle.

	TW (GHB A)	TW (GHB B)	BW (GHB A)	BW (GHB B)
T	(N=15) 40.01±2.28	(N=19) 40.65±4.44	(N=15) 0.86±4.5508E-02	(N=19) 0.83±4.6202E-02
	n.s.	n.s.	n.s.	n.s.
WV	(N=19) 40.40±5.28	(N=19) 40.40±5.28	(N=19) 0.82±7.7808E-02	(N=19) 0.82±7.7808E-02

All values are expressed as means ± SD. N: number in sample; n.s.: not significant; T: Treated; WV: Water vehicle; TW (GHB A): Total body weight of chicks treated with dose A; TW (GHB B): Total body weight of chicks treated with dose B; BW (GHB A): Brain weight of chicks treated with dose A; BW (GHB B): Brain weight of chicks treated with dose B.

Results and discussion

The measurements obtained on day 21 of incubation in the vehicle-injected chicks were compared with those obtained in chicks treated with dose A at 7 and 11 days of incubation. TD and VD measurements were statistically higher ($p<0.001$) in the GHB-treated chicks than in the vehicle-injected controls. However, the APD did not differ significantly between either groups.

With respect to dose B (Table 2), significant differences were again found for the TD ($p<0.001$) and VD ($p<0.01$), which were greater in the treated chicks.

The alteration in the TD and DV parameters suggests a distorting action of the drug, which would elicit a disproportion in cranial development. This can be explained in terms of the active development of the acrochordal cartilage and otic and orbitary capsules from days 6 and 7 of incubation, which continues until after day 11. This development clearly influences the transversal and vertical diameters of the cranium. The absence of significant differences in total or brain weights between the vehicle- and GHB-injected chicks (Table 3) rules out any influence in changes in measurements due to variations in such weights. We are currently unable to explain why smaller doses produce greater effects.

In the literature, there are reports on the therapeutic effects of GHB in patients with brain lesions (Snead et al., 1989; Volpi et al., 2000), and human and experimental studies on its biochemical characteristics (Couper and Logan, 2000; Vergoni et al., 2000; Okun et al., 2001; Itzhak and Ali, 2002), including electroencephalographic effects (Van Sassenbroeck et al., 2001) and effects on the cortex or central nuclei (Jensen and Mody, 2001). However, we have found no reference to the effects of its action on embryonic tissues. We therefore have no studies with which to compare our findings.

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