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

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SUMMARY

Gamma-hydroxybutyrate (GHB), a precursor to gamma aminobutyric acid (GABA), was initially employed as an anesthetic. As a relatively novel drug, few people are aware of its harmful effects and few studies have been undertaken to investigate its long-term effects or its action on developing tissues.

We performed an experimental study on the action of GHB on the developing eye, an organ very closely related to the development of the CNS. Chick embryos were treated with 20% or 30% dilutions of 100 µl GHB at 7 (30-31 HH) and 11 (37 HH) days of incubation (i.e., two doses per group), and the effects were observed at 21 days of incubation (45 HH). An ophthalmologic ultrasonography device (Hondex A/B SCAN IS-500) was used to measure different eye parameters (corneal thickness; posterior surface of cornea – anterior surface of lens; anteroposterior diameter of lens; anteroposterior diameter of eye).

We observed significant differences between the treated and control groups as regards the thickness of the cornea and lens, and in the anteroposterior diameter of the eye. The present results demonstrate a possible effect of GHB on development.

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

INTRODUCTION

Gamma-hydroxybutyrate (GHB) was first synthesized in France 34 years ago. It is a precursor in the metabolism of the neurotransmitter gamma aminobutyric acid (GABA), obtained by replacing the amino group with a hydroxyl group. Unlike GABA, GHB can cross the blood-brain barrier.

GHB and GABA have some characteristics in common, although there is debate about whether GHB is an agonist of GABA (Feigenbaum et al., 1996). GHB produces wider effects and is a potent central nervous system (CNS) depressant, increasing the concentration of dopamine in the brain (Dzoljic et al., 1975) and producing a strong feeling of euphoria and alertness (Engelsen and Christensen, 1999; Strange and Jensen, 1999).

Initially, GHB was found to be of value in the treatment of patients with narcolepsy, drug dependence (Poldrugo and Addolorato, 1999), and brain injury (Maslov et al., 1987; Volpi et al., 2000). However, it was found to produce hallucinations and confused states

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when used as an anesthetic, and its use for this purpose was discontinued. Later, when the first reports of intoxication demonstrated its potential use as a drug of abuse, it became known as «liquid ecstasy».

Awareness of its euphoric effects, which can last for about an hour, has led to it becoming one of the most widely used drugs, especially because it is, unfortunately, cheaper than any other recreational drug, including ecstasy. It can produce initial symptoms of somnolence, hypotonia, confusion, headache, and loss of balance, and its effects may subsequently progress to a coma, with severe respiratory alterations secondary to CNS depression (Ingels et al., 2002; Miotto et al., 2001; Nini et al., 2001; Feldman and Croquette-Krokar, 2002).

Because GHB is a natural component in the metabolism of mammals, individuals may react differently, making its effects difficult to predict. Disturbingly, because it is odorless, almost tasteless, and water-soluble, it can be put into a drink without the drinker noticing. Thus, it can be taken in association with alcohol or any other drug with even more harmful effects (Degenhardt et al., 2002; Knudsen et al., 2008). Furthermore, it is impossible to measure out a precise dose of the liquid in the setting where it is habitually consumed.

GHB is a relatively novel drug, such that few people are aware of its harmful effects, and few studies have been undertaken to investigate its long-term effects or its action on developing tissues. Because consumers tend to be in fertile age groups, studies of the effects of GHB in development are of special interest (Torres de Galvis, 2003). Because GHB is a precursor to GABA, its action on the CNS largely occurs when this tissue is being formed.

Here, we performed an experimental study on the effect of GHB on the developing eye, which is closely related to the development of the CNS. Days 7-11 of incubation (30-31 HH) and (37 HH) in chicks (Hamburger and Hamilton, 1951) are also key dates for the development of the eye (see below).

The development of the eye in the chick embryo is comparable to that observed in mammals, including humans, and its different layers derive from the same embryonic sheets. This similarity persist in the adult, making the avian eye a good experimental model to determine possible alterations of the human eye as a result of different situations or agents. The retina, the nervous part of the eye, is formed by the optic vesicle, a prolongation of the diencephalic vesicle. Correct development of the eye is known to depend on the presence of this nervous tissue. The interior of the eye has two compartments: an anterior chamber, between the cornea and the lens, and a posterior chamber, between the lens and the retina, which is covered by the choroid coat, itself covered by the sclera. We therefore selected as reference points for our study the cornea, lens, vitreous humor, and retina. We considered the Romanoff (Romanoff, 1960) calendar of these elements. The development of the cornea begins at day 7 of incubation. On this day, a change occurs in the position of crystalline fibers in the area between the epithelium and the body of the lens. By day 8, the central part of the nucleus starts to degenerate, suggesting that this may influence the dimensions of the lens. The development of the vitreous body commences at around day 4. The zonula and its definitive structure are not observed until day 14, such that between days 7 and 11 they are in full development.

The development of the retina (reorganization and cell differentiation) takes place as from day 8. The first sign of the internal reticular layer appears at days 7-8. By day 8, the ganglion layers are present, except in the marginal quarter. After days 8 or 9, the rows of nuclei in the ganglion layers grow threefold, and the thickness of the retina increases until day 10. Cones and rods begin to differentiate between days 10 and 12. The area of maximum visual acuity develops later, and its development does not end until hatching. Among other events that may influence the development of the eye in this period is the inclusion from day 7 of vessels in the optic vesicle, after partial closure of the choroid fissure. The true cartilaginous sclera appears on days 8-9, with the capillary layer that will constitute the choroids separated from the sclera.

Our study focused on the effects of the toxic agent on the interior of the eye and its different elements during a period of development of great interest.

Material and Methods

Reagents and supplies

We incubated 180 fertilized Leghorn HR7 eggs, weighing 55-65 g, at 37.8º ± 0.4º C at a relative humidity of 60-70 % in a Masalles

Model 65 incubator equipped with forced ventilation and automatic voltage. The GHB used was produced according to the Kitchen Optimized GHB Synthesis formula (http://www.lycaeum.org/drugs/synthetics/ghb/kitchen.html). This formulation was selected for the study because it is the most widely used in the market. Measurements were performed using a Hondex A/B SCAN IS-500 ophthalmologic ultrasonography apparatus.

Treatment

The eggs were divided equally between four groups: one group was injected only with vehicle (100 µl distilled water); another with dose A (100 µl GHB at 20% dilution), and a third with dose B (100 µl GHB at 30% dilution). The eggs were injected on days 7 and 11 of incubation, (30-31 and 37 H.H.) (Hamburger and Hamilton, 1951) (i.e., two doses per group), which are key dates in embryonic development (Romanoff, 1960). The fourth group was left untouched as controls. Sterile distilled water was selected as the ideal vehicle, because we found it less harmful than physiologic serum (Table 1). The injection was always made into the air chamber at the larger end of the egg, making two 1-mm holes through the cuticle, shell, and 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.

Table 1. Effects of distilled water vehicle and serum vehicle on eye parameters of the chick embryo (in mm) in relation to parameters of controls.

	CT	CL	APL	APE
WV	(N=19) 1.1895±0.1915	(N=19) 1.2±0.1915	(N=19) 1.6368 ±0.1571	(N=19) 8.7579±0.3355
	p<0.01	n.s.	p<0.01	n.s.
C	(N=19) 1.0947±0.1408	(N=19) 1.0263±0.1408	(N=19) 1.2895±3.153E-02	(N=19) 8.6368 ±0.3353
	p<0.01	p<0.05	p<0.001	n.s.
SV	(N=20) 1.19±9.679E-02	(N=20) 1.29±0.1619	(N=20) 1.55±0.1357	(N=20) 8.8050±0.1669

All values are expressed as means ± SD. N: number in sample; n.s.: not significant; WV: Water vehicle; C: Controls; CT: Corneal thickness; CL: Posterior surface of cornea – anterior surface of lens; APL: Anteroposterior diameter of lens; APE: Anteroposterior diameter of eye.

The animals were sacrificed with ether anesthesia, on day 21 of incubation (46 H.H.) (Hamburger and Hamilton, 1951). Fifteen, 19, or 20 eggs were taken from each group, depending on the mortality rate in the group, for the measurement of the following representative eye parameters: corneal thickness (CT); distance between the posterior surface of cornea and the anterior surface of lens (CL), anteroposterior diameter of the lens (APL), and anteroposterior diameter of the eye (APE).

Analyses

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

Results

The measurements obtained on day 21 of incubation (46 HH) (Hamburger and Hamilton, 1951) in vehicle (distilled water) – injected chicks were compared with those obtained in chicks injected with dose A (20% GHB) at 7 and 11 days of incubation, (30-31 and 37 HH), (Hamburger and Hamilton, 1951) (Table 2). Significant differences were observed in the CL ($p<0.05$), APL ($p<0.01$), and APE ($p<0.01$) measurements, treated chicks having a smaller CL and APL, and a larger APE. There was no significant difference in corneal thickness measurements (CT).

Table 2. Effects of GHB (dose A and dose B) on eye parameters of the chick embryo (mm).

	CT	CL	APL	APE
Treated (dose A 20%GHB)	(N=15) 1.1467±0.1457 n.s.	(N=15) 1.0633 ± 0.1280 p<0.05	(N=15) 1.3667±0.3155 p<0.01	(N=15) 9.1133±0.3962 p<0.01
Vehicle-injected	(N=19) 1.1895±9.366E-02	(N=19) 1.2000 ± 0.1915	(N=19) 1.6368±0.1571	(N=19) 8.7579±0.3355
Treated (dose B 30%GHB)	n.s. (N=19) 1.1316±9.459E-02	p<0.01 (N=19) 1.0105 ± 0.1243	p<0.001 (N=19) 1.2211±0.1782	n.s. (N=19) 8.9526±0.3596

All values are expressed as means ± SD. N = number in sample, n.s.: not significant; CT: Corneal thickness; CL: Posterior surface of cornea to anterior surface of lens; APL: Anteroposterior diameter of lens; APE: Anteroposterior diameter of eye.

Highly significant differences in CL ($p=0.001$) and APL ($p<0.001$) measurements were recorded between dose B (30% GHB) – treated and vehicle-injected chicks at 21days of incubation (46 HH) (Hamburger and Hamilton, 1951) (Table 2), the treated chicks showing smaller values. However, there were no significant differences in the CT or APE measurements.

Discussion

Our results indicate that dose A (20% GHB) affected the development of the lens, whose structure undergoes organization during this period. The thickness of the lens was reduced, as was the size of the anterior chamber, but there was no effect on corneal thickness. This suggests a displacement of the lens toward the anterior part of the eye. The anteroposterior dimension of the eye increases at the expense of the posterior chamber, where the vitreous body is in full development, and at the end of which the retina is developing and being structured, as commented above.

The reduction in lens thickness and anterior chamber was even more significant with dose B (30% GHB). However, there was no difference in the corneal thickness or anteroposterior diameter of the eye between the dose-B and vehicle-treated chicks. We are currently unable to explain the normal values of the anteroposterior length of the eye in the chicks treated with this dose.

The literature contains reports on the therapeutic effect of GHB in patients with brain injuries (Snead et al., 1989; Okun et al., 2001), and human and experimental studies addressing the biochemical characteristics of GHB have been published (Couper and Logan, 2000;Vergoni et al., 2000; Okun et al., 2001; Itzhak and Ali, 2002; Carter et al., 2003), including electroencephalographic effects (Van Sassenbroeck et al., 2001), effects on the cortex or central nuclei (Jensen and Mody, 2001), acute and subchronic effects on aggression (Navarro et al., 2007), and effects other than those of ethanol (Baker et al., 2008), etc. However, we found no reference to the effects of its action on embryonic tissues.

Previous experiments by our group (Martín-Molina, 1995), have demonstrated that another drug, alcohol, has a clear effect on eye development, with a reduction in both lens size and the thickness of retina in the ganglion cell layer, resulting in a reduction in the number of optic nerve fibers; the thickness of the corneal layer is also decreased. In the present study, eye development was also affected by GHB, pointing to the sensitivity of this organ to neurotoxic agents. However, such effects are not identical, because although GHB also reduced the thickness of the lens in a clearly significant manner, there was no alteration of the corneal layer. As stated above, we have found no studies in the literature concerning the effects of GHB on embryonic tissues, precluding a comparison of our findings with others. The present results are of interest because the observed effects suggest that this drug may produce changes in the normal proportions of the eye and, therefore, in its functionality.

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