HPLC-Determination of Apomorphine in a Mormyrid Fish, Gnathonemus petersii

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Uptake of apomorphine and elimination kinetics in brain and muscle tissue of the weakly electric fish *Gnathonemus petersii* (Mormyridae) was determined by HPLC. 20 min exposure of the fish to apomorphine in the aquarium water (0.4 mg/l) resulted in a concentration factor of 1.09 for brain and 0.55 for muscle tissue. Elimination from brain tissue can be described with first order kinetics ($t_{1/2} = 2.4$ h).

Introduction

The electric behavior of the weakly electric fish *Gnathonemus petersii* (Mormyridae) is influenced by dopaminergic drugs [1], with the DA-agonist apomorphine and the DA-antagonist haloperidol producing opposite effects. The effects of both substances become apparent 15 min after application. Thus, a fast uptake, or low threshold, has to be assumed. In the present paper the accumulation of apomorphine in brain tissue (and in comparison also in muscle tissue) of G.p. after application to the aquarium water and the elimination of apomorphine is investigated using the HPLC technique of Westerink and Horn [2].

Materials and Methods

Apomorphine-hydrochloride was obtained from Sigma Chemical, St. Louis, U.S.A. HPLC-water and methanol (Merck, Darmstadt, F.R.G.) were HPLC-grade, all other chemicals were analytical grade or better and used as received. A standard solution of apomorphine-hydrochloride (1 g/l) in ascorbic acid (2 g/l) was freshly prepared every day.

The mobile phase consisted of methanol and an aqueous solution of 0.02 M di-sodium hydrogen phosphate dihydrate and 0.04 M citric acid hydrate, pH 3.0 (40:60). It was degassed ultrasonically before use. An LKB 2150 pump was used at a flow rate of 0.7 ml/min. Samples were injected *via* 200 µl loop. Separation was achieved by a Macherey-Nagel Nucleosil 100 5 C18 column (250 mm × 4 mm I.D.) in combination with a Nucleosil 100 10 C18 precolumn. The electrochemical detector (ERC BCMA JN I) contained a glassy carbon working electrode (maintained at an applied potential of +0.7 V), a Ag/AgCl reference electrode, and a stainless steel auxiliary electrode.

Gnathonemus petersii (ranging in length from 8 to 15 cm) were obtained from a petshop and kept in a 200 l aquarium at a temperature of 25 to 28 °C. The fish were fed on frozen mosquito larvae. Two days before treatment a fish was transferred into a separate aquarium filled with 15 l of water; it contained a hiding place for the fish. 6.0 mg of apomorphine-hydrochloride were dissolved in 6 ml of ascorbic acid solution (100 g/l). This solution was carefully added to the 15 l aquarium water resulting in a final apomorphine concentration of 0.4 mg/l. After 20 min exposure to this concentration, a fish was either killed immediately by cutting the rostral end of the spinal cord or transferred to an aquarium with fresh water, where it remained for different lengths of time (2, 4, and 8 h) and was killed then. For each case a set of six fish of about the same length was used, but each fish was treated separately.

An external standard was used because an appropriate internal standard (N-*n*-propylnorapomorphine) was not available to us. For calibration known concentrations of apomorphine-hydrochloride (5 µg/l to 30 µg/l) were prepared by diluting the standard solution, treated the same way as tissue samples, and chromatographed. Peak areas and calibration curve were calculated using LKB-Nelson software resulting in a regression line with a correlation factor of 0.991 (Fig. 1). Recovery was determined by adding known amounts of apomorphine to brain and muscle tissue and processing the samples as above.

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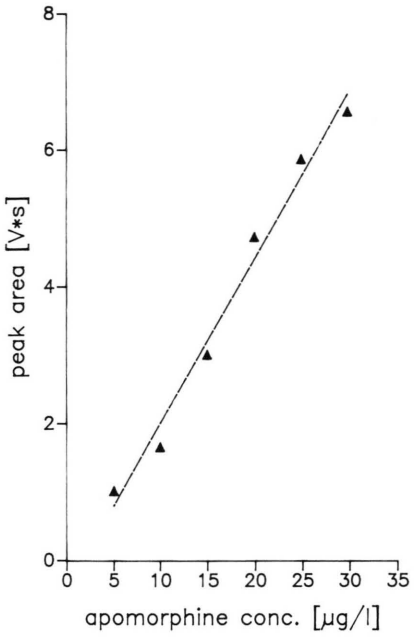

Fig. 1. Calibration curve (correlation factor 0.991) showing peak areas *(V*s)* of known apomorphine concentrations (µg/l). The curve shows good linearity between 5 µg/l and 30 µg/l.

Immediately after death the fish's head capsule was opened and the brain removed. Also about 100 mg of paraxial muscle was excised. The samples were weighed and put on ice immediately. To each sample 0.4 M perchloric acid at an amount of 1 ml/100 mg tissue was added and the sample homogenized ultrasonically. For precipitation a KOH/formic acid solution was added at 0.1 ml/ml perchloric acid. This solution consisted of 15 ml concentrated formic acid (98%) which was added carefully to 50 ml of 10 M potassium hydroxide. The homogenates were centrifuged (20 min, $4000 \times g$) and the supernatants directly injected. The resulting peak areas were converted into apomorphine concentrations (µg/l) by means of the calibration curve.

Results

The lower limit of apomorphine detected in brain and muscle tissue with the described method was 5 µg/l. Concentrations below this value were within the noise of the baseline and used as 0 in the calculations. Control fish not treated with apomorphine never showed a peak at the apomorphine retention time.

In brain tissue the determined apomorphine concentrations ranged from 3 to 30 µg/l. In the following they are presented as pg/mg tissue (± S.D.). The mean apomorphine level decreased, in a non-linear way, from 185.5 (± 52.3) pg/mg at 0 h of treatment to 30.0 (± 16.9) pg/mg at 8 h after treatment (Fig. 2).

Non-linear regression using a function of the form $y = ae^{-kt}$ for first order kinetics yielded a starting concentration $a = 181.1$ pg/mg, an elimination factor $k = 0.288$ and a correlation factor of 0.981. Half-life of apomorphine $t_{1/2} = (\ln 2)/k$ for brain tissue was 2.4 h (144 min). Analysis of variance showed that the 4 mean values are not identical (significance level 95%). Regarding the determined apomorphine recovery of 41.5%, the actual concentration of apomorphine in brain tissue at 0 h after treatment can be calculated to be 436.4 pg/mg, correspondingly 0.436 mg/l. Compared to the apomorphine concentration of 0.4 mg/l aquarium water, the resulting concentration factor is 1.09.

In muscle tissue the determined apomorphine concentrations were between 3 and 10 µg/l, *i.e.* close to or below the detection limit. The corresponding mean tissue concentrations (Fig. 3) are 62.3 (± 34.5) pg/mg at 0 h after treatment and 35.0 (± 6.9) pg/mg

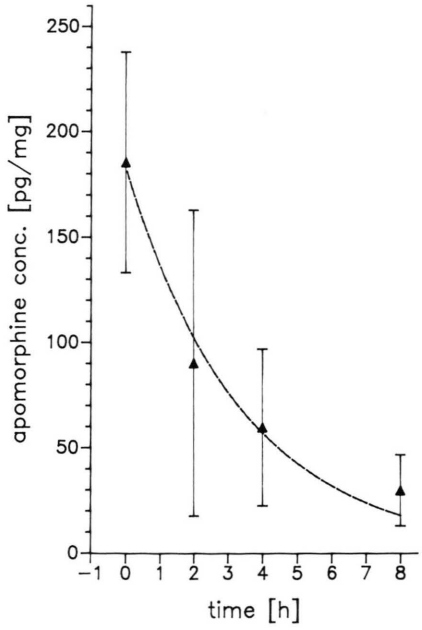

Fig. 2. Time course of apomorphine in brain tissue of *G. p.* The plot shows mean values ($n = 6$) and standard deviations at different lengths of time after treatment of the fish and the best fit of the function $y = ae^{-kt}$.

at 8 h. Although non-linear regression analysis (correlation factor 0.771) suggests a decrease during 8 h, analysis of variance showed no difference between mean values. The concentration factor of 0.55 determined as above is much lower than in brain tissue.

Discussion

The HPLC-method of Westerink and Horn [2] was applied to fish tissue because this method is fast, easy to handle, and shows sufficient and reproducible results even by using external standard calculation. (An extraction of apomorphine by ethyl acetate showed a co-extracted tissue component that interfered with the apomorphine peak.)

Apomorphine is present in the brain of *G.p.* with a concentration factor of 1.09 after 20 min of treatment. This finding is in good agreement with the reported change of electric behavior which is maximal within 15 to 30 min after application [1]. Higher concentration in the brain compared to muscle might be due to the presence of specific binding sites of the dopaminergic system. The determination of brain apomorphine concentration might have resulted in too high a concentration. Because of the blood-brain-barrier apomorphine in the brain blood vessels would positively contribute to the apomorphine concentration of brain tissue as long as invasion is going on. Unfortunately, we did not succeed in obtaining sufficient amounts of blood for apomorphine determination due to the minuteness of the vessels of our fish. Assuming a relative blood volume of 4% for the total brain, the error should be small.

The decrease of the apomorphine level in brain of *G.p.* follows an exponential time course of the form $y = ae^{-kt}$. This result suggests that the elimination of apomorphine is an undivided process. The half-time of 144 min was calculated assuming a one-compartment model. Such a model was also used by Bianchi and Landi [3] for the kinetic profile of apomorphine in rat brain. They determined a half-life time of 11 min. An explanation for the differences between the warm blooded rat and the cold blooded fish solely based on temperature differences would be unsatisfactory. Even if this difference is accounted for using a Q_{10} of 2–3, the half-life in the fish would exceed that in the rat considerably. Thus, different elimination processes in fish and rat have to be assumed. For the fish the elimination pathway is yet unknown.

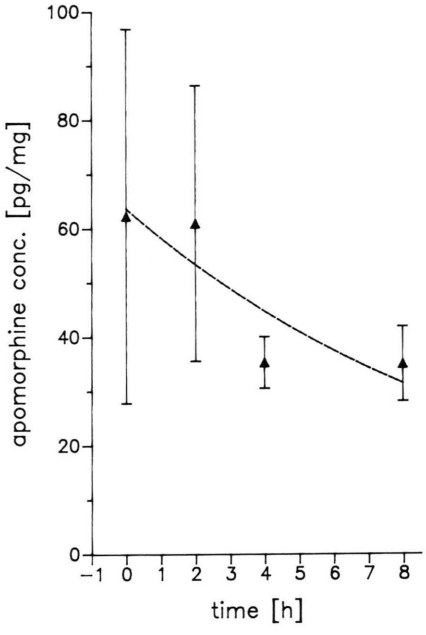

Fig. 3. Mean values and standard deviations of apomorphine concentrations in muscle tissue of *G.p.* at different lengths of time after treatment and best fit of the function $y = ae^{-kt}$.

[1] P. Kunze, H.-U. Wezstein, Z. Naturforsch. **43c,** 105–107 (1988).
[2] B. H. C. Westerink, A. S. Horn, Europ. J. Pharm. **58,** 39–48 (1979).
[3] G. Bianchi, M. Landi, J. Chromatog. **338,** 230–235 (1985).