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A tryptamine analog with high affinity to the heart tissues is a potential antiarrhythmic agent

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Abstract

A novel tryptamine analog, 1-methyl-3-[*N*-(3-indolyl)ethyl]carbamoyl-1,4-dihydropyridine (T-CDS) was synthesized and converted into a stable, solid complex with 2-hydroxypropyl-β-cyclodextrin. An aqueous solution of the complex was given intravenously to dogs and the concentration of T-CDS and its corresponding quaternary (T-Q^+) forms were monitored in the blood for 50 min. The effect of the drug on vital heart parameters was monitored throughout the studies. At the end of the experiment the dogs were sacrificed and the concentration of the quaternary pyridinium form (T-Q^+) was determined in the different heart tissues, as well as in the kidney, liver, lung, brain, urine and cerebrospinal fluid. The compound was found to be selectively bound to the heart muscles and showed different concentrations in different heart tissues. The T-Q^+ concentrations were much higher in the heart after administration of the dihydro form (T-CDS), than after administering T-Q^+ directly. The compound was found to be active on certain vital signs of the cardiovascular system and could be an effective and safe antiarrhythmic agent.

Introduction

Tryptamine (T, **1**; Figure 1) is an indolealkylamine which is biosynthetically derived from the essential amino acid tryptophan. Tryptamine is present in very low endogenous concentrations. The application of a redox targeting system for enhanced brain-targeted delivery of this amine, based on the 1,4-dihydrotrionelline ↔ trigonelline redox pair was previously reported (Bodor et al 1986). Briefly, tryptamine is converted into its corresponding trigonellylamide (T-Q^+, **3**), which is reduced to the 1,4-dihydrotrigonellylamide derivative, which is called a chemical delivery system (T-CDS, **4**). When administered intravenously to animals, it was shown that the lipophilic T-CDS is distributed throughout the body, including the brain, followed by enzymatic oxidative conversion by the ubiquitous $NAD^+ \leftrightarrow NADH$ coenzyme system, into the hydrophilic T-Q^+. This, in turn, is quickly eliminated from the body, but the portion formed in the brain is locked-in by the blood–brain barrier, providing a long-term presence of T-Q^+, which subsequently is slowly cleaved to tryptamine, providing its sustained release. This redox system was shown to provide enhanced and targeted delivery of various therapeutic agents to the CNS in a specific and sustained fashion (Bodor et al 1981; Bodor & Buchwald 1999). Examples include dopamine (Bodor & Farag 1983; Simpkins et al 1985; Omar et al 1994), estradiol (Bodor et al 1987; Simpkins et al 1986; Estes et al 1987), penicillins (Pop et al 1989), antiepileptics

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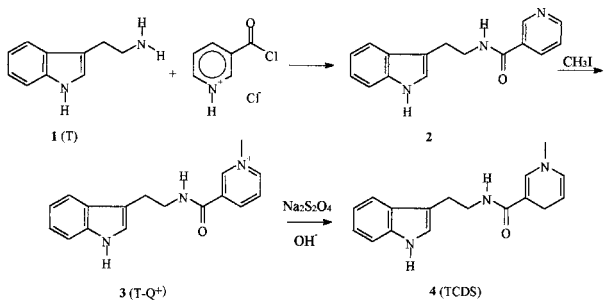

Figure 1 Synthesis of the tryptamine chemical delivery system (T-CDS) from tryptamine (**1**). T-CDS is obtained in the last step by chemical reduction of its quaternary pyridinium precursor ($T\text{-}Q^+$). This process is reversed in-vivo, as after administration, the T-CDS is oxidized enzymatically, everywhere in the body, to the quaternary derivative $T\text{-}Q^+$ (**3**).

(Pop & Bodor 1992) and neuropeptides (Bodor et al 1992; Prokai et al 1994; Prokai-Tatrai et al 1996; Chen et al 1998). In all these cases, the drug-Q^+ conjugate was found to be present in the brain at higher concentrations than in all other organs lacking the equivalent of a blood–brain barrier. However, the tryptamine redox system behaved very differently. While the brain lock-in took place, as in all the other cases, providing higher and more sustained concentration of the $T\text{-}Q^+$ complex in the brain than in the blood, it was found that in the rat $T\text{-}Q^+$ was present at much higher concentrations in the heart than any other organs. This was the case when T-CDS was administered, or when the oxidized form, $T\text{-}Q^+$, was given. The difference was that the $T\text{-}Q^+$ concentrations in the heart were lower when it was administered as such, which can be explained by the very large orders-of-magnitude difference between the lipophilicity–tissue penetration properties of T-CDS vs $T\text{-}Q^+$. Consistent with this, $T\text{-}Q^+$ could not be found in the brain following its administration. But the concentrations in the heart were still larger than in the other organs. It is evident from these results that the heart tissues have specific affinity for $T\text{-}Q^+$. This phenomenon of specific uptake in the heart, which has not been observed in similar redox systems thus far investigated, was unexpected.

This work had the main objective of confirming this unusual distribution in another species, the dog, and if it was confirmed, to study the effect of $T\text{-}Q^+$ on cardiac electrophysiology. The T-CDS was formulated as the stable solid complex with 2-hydroxypropyl-β-cyclodextrin, which was used for the preparation of the injectable solutions in sterile saline.

Materials and Methods

Chemistry

All chemicals were reagent grade and bought from Aldrich or Sigma. Solvents used for high-performance liquid chromatography were HPLC grade obtained from Fisher Scientific. Melting points were determined with a Fisher-Johns apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlab Inc. (Norcross, GA). The HPLC system consisted of Spectra Physics SP8810 pump, SP8450 detector operated at 254 nm, SP4290 integrator and Reodyne 7125 injector with a 20-μL loop. Separation was performed on a Waters RCM 8×10 RP (phenyl) cartridge column, at a flow rate of 2 mL min^{-1} with a mobile phase consisting of acetonitrile–0.05 M $(NH_4)_2HPO_4$ (35:65) and contained 0.003% octanesulfonic acid sodium salt.

Synthesis

*3-{*N*-[2-(3-indolyl)ethyl]carbamoyl}pyridine* (**2**)
To a solution of 1.8 g (10 mmol) of nicotinoyl chloride hydrochloride in 10 mL of pyridine, 1.60 g (10 mmol) of tryptamine was gradually added while stirring. The mixture was left at room temperature, while stirring, overnight, then distilled on rotavap under reduced pressure. The residue was dissolved in 25 mL of ethyl acetate, washed with water, dried with anhydrous sodium sulfate and distilled. The residue was crystallized from isopropanol to give 2.1 g (81%) of white solid of mp 150–152°C, as reported previously (Bodor et al 1986).

*1-Methyl-3-{*N*-[2-(3-indolyl)ethyl]carbamoyl}pyridinium iodide (T-Q^+, **3**)*
To a solution of 1.6 g (6 mmol) of compound 2 in 20 mL of acetone, 2 mL of iodomethane was added and the mixture was refluxed overnight, while stirring. The fine yellow solid separated was filtered, washed with acetone, dried and crystallized from methanol–isopropanol mixture to give 2.0 g of a solid with mp 215–217°C as reported (Bodor et al 1986). FAB-MS: 280.5 (Q^+), 688.4 ($2Q^+ + 1^-$).

*1-Methyl-3-{*N*-[2-(3-indolyl)ethyl]carbamoyl}-1,4-dihydropyridine (T-CDS, **4**)*
To a suspension of 1.9 g (4.7 mmol) of finely powdered compound **3** in 150 mL of deaerated water, 150 mL of ethyl acetate and 3.2 g (32 mmol) of $NaHCO_3$ were added. The mixture was cooled in an ice bath and 5.3 (25.8 mmol) of $Na_2S_2O_4$ was gradually added while stirring. The mixture was flushed with nitrogen and left stirring for 6 h. The organic layer was separated, washed

with water, dried with anhydrous sodium sulfate and distilled on rotavap under reduced pressure to give 1.3 g (65% yield) of pale-yellow solid of mp 50–55°C. The compound was identified by ^{1}H NMR and FAB-MS (Bodor et al 1986).

T-CDS complex with hydroxypropyl-β-cyclodextrin
Method A: solid solubilization. To an ice-cold solution of 12.0 g of 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) in 12 mL of water, compound **4** was added in 0.1-g portions, while sonicating the mixture, until no more solid seemed to dissolve. The mixture was kept under a stream of nitrogen all through the experiment. The mixture was then filtered and the filtrate was lyophilized. A yellowish-white solid was obtained which was shown by HPLC to contain 29.4 mg T-CDS (g solid complex)$^{-1}$.

Method B: co-solvent solubilization. A solution of 1 g of **4** in 25 mL of absolute ethanol was gradually added, while sonicating, to a solution of 25 g of HP-β-CD in 50 mL of water. The solvents were evaporated in vacuum (rotavapor) and the glassy residue was reconstituted in 50 mL ultrapure water. The solution was filtered through a 0.45-μm hydrophilic membrane, and lyophilized (Labconco Freeze Dryer Model 18). The amount of **4** incorporated into the complex was determined by HPLC to be 41.3 mg g^{-1}.

Pharmacology and distribution studies

Solution for injection
Solid T-CDS complex in HP-β-CD was dissolved in a volume of sterile saline solution equal to its weight. T-Q$^+$ was dissolved in a mixture of 25% ethanol, 25% propylene glycol, and 50% sterile saline solution, keeping the volume of solvent to the minimum necessary for solubilization.

Dog preparation
Four mongrel dogs of either sex, weighing 20–25 kg, were anaesthetized with sodium pentobarbital (35 mg kg^{-1}, i.v.) and intubated with a cuffed endotracheal tube. The dogs were ventilated with room air using a Harvard animal respirator and their body temperature was monitored and maintained at 37–38°C.

Electrode catheters (Fr. 5 and 6) were introduced via the right femoral artery and veins. The electrode catheters were positioned under X-ray and intracavitary ECG control in the following positions: high right atrium (HRA) to record high right atrial electrical activity and to pace HRA; aortic root, to record His bundle (HBE) activity; right ventricular apex, to record and stimulate the ventricle; and pulmonary artery (Elecath thermodilution catheter into one dog), to measure the cardiac output (CO). Standard surface ECG leads were recorded with subcutaneous needle electrodes. One of the right leg veins was cannulated for drug administration, and the left femoral vein was cannulated for blood withdrawal. After insertion of catheters, 30 min was allowed for equilibration before measurements. Blood samples were taken at regular intervals for drug-level monitoring. Data were recorded using a GOULD TA 2000 eight-channel recorder. Pacing was accomplished with a battery-powered universal heart stimulator (Biotronik UHS 20). The CO was measured by Elecath Cardiac Ouput Computer. At the end of the experiment, the anaesthetized dogs were rapidly infused with pentobarbital into the femoral artery, until death occurred. At this time the thorax, abdomen and skull were opened and tissue specimens were taken from the left ventricle of the heart, and from different parts of the lung, kidney and brain, in addition to samples of blood, urine and cerebrospinal fluid (CSF).

Measurements
The following parameters were measured: sinus cycle length (SCL); atrial and antiventricular conduction time (AH); and His-Purkinje conduction time (HV).

Using programmed electrical stimulation, sinus node recovery time (SNRT), sino-atrial conduction time (SACT), and atrial, AV nodal, His-Purkinje system (HPS) and ventricular functional and effective refractory periods were also measured and calculated. Measurement of SNRT involved pacing the right atrium for more than 30 s followed by abrupt termination; the interval from the last paced complex to the onset of the first sinus beat as measured from the right atrial electrogram was the SNRT (Mandel et al 1971). This interval was corrected for the underlying sinus rate to give corrected SNRT (CSNRT). The SACT was an estimate of conduction time into, and out of, the sinus node through the perisinusoidal region. We used the Narula method for estimating SACT (Narula et al 1978). In this technique, the atrium was paced for eight beats, 10 beats min^{-1} faster than the underlying sinus rate (110–120 beats min^{-1}). The interval from the last paced beat to the first return sinus beat, recorded in the high right atrium, was measured. The SACT was the interval minus the mean sinus cycle length. Atrial, AV nodal, HPS and ventricular functional and effective refractory periods were determined by the extramulus technique at con-

stant atrial and ventricular driven cycle length (Mandel et al 1971). Atrial and ventricular programmed stimulation was performed as follows: at every 8th basic stimulus (S1), a test stimulus (S2) of the same intensity and duration was delivered at a progressively decreasing S1–S2 interval (10-ms steps) until S2 failed to evoke a propagated response. Each test stimulus was followed by a 5-s pacing inhibition before the next series of test stimulus. The definition of refractory periods were those of Wit et al (Wit et al 1970). The diastolic threshold of the right atrium and ventricle were determined at the beginning and the end of each experiment.

After basic measurements were taken, the dogs were administered different doses of T-CDS and T-Q^+. One dog (no. 1) was given a loading dose (i.v. bolus dose of 20 mg kg^{-1}) followed by 10 mg kg^{-1}. Two dogs (nos 2 and 3) were injected intravenously with 20-mg-kg^{-1} doses. One dog (no. 4) was given intravenous doses of 2 mg kg^{-1} and, later, 10 mg kg^{-1} of T-Q^+. None of the dogs received both compounds. After injection of the compounds, the ECG was continuously recorded, and the measurements were repeated 10 min after the injection.

Distribution

At 2, 5, 10, 15, 25, 35 and 50 min, a sample (1 mL) of blood was withdrawn and added immediately to a teared tube containing 4 mL of the extraction solvent (5% DMSO in acetonitrile), shaken and immediately cooled in ice and centrifuged. After performing the electrophysiology studies the anaesthetized dog was injected with pentobarbital as rapid infusion in the femoral artery until death occurred. Specimens from heart tissue, lung, kidney, liver and different brain regions, in addition to samples from CSF and urinary bladder, were collected. The CSF and urine samples were treated with four times their volume of the extraction solvent, shaken, cooled and centrifuged. The solid tissue samples were homogenized with equal amounts of their weight of water and then treated with four times their weight of the extraction solvent, shaken and centrifuged.

Results and Discussion

Chemistry

T-CDS (**4**) was prepared using a modification of the reported (Bodor et al 1986) method (Figure 1). The T-CDS complex with HP-β-CD was prepared so that the compound could be administered in saline to dogs while increasing the stability of the compound against oxidation before and during handling of its solution. Two methods were followed to prepare the complex: the solid and the co-solvent methods. A higher concentration of T-CDS in the complex could be obtained by the co-solvent method (41.3 mg g^{-1} vs 29.4 mg g^{-1}).

Distribution

After intravenous injection of an aqueous solution of T-CDS complex with HB-β-CD at a dose of 20 mg kg^{-1} dog body weight, no intact T-CDS could be detected in the blood at any of the time points and only T-Q^+ was detected at a concentration of 26.7 μg mL^{-1}, 2 min post injection. The concentration then rapidly decreased to reach 5.4 μg mL^{-1} within 50 min (Figure 2). Calculation of the pharmacokinetic parameters resulted in C_o = 33.3 μg mL^{-1} (extrapolation to t = 0), theoretical volume of distribution = 13.2 L and $t_{\frac{1}{2}}$ for clearance = 22.7 min. The concentration in the heart tissues 50 min post injection, when compared with the concentrations in other organs and fluids, showed that T-Q^+ preferentially binds to the heart tissues (Figure 3). The average T-Q^+ concentration in the heart tissues at 50 min was about 40 times that in blood, and more than 3 times that in brain tissue. The concentration of T-Q^+ was also found to vary in different heart tissues, which may reflect differences in tissue specificity, blood supply or rates of T-CDS $\rightarrow$ T-Q^+ conversion. No blood samples were collected from the dog injected with T-Q^+, but on analysing its heart tissue samples, T-Q^+ was found in much lower concentrations than when T-CDS was injected and there was not much difference between its concentrations in different heart tissues. Accordingly, injecting the lipophilic dihydro compound is necessary for deep distribution in the heart muscles followed by oxidation to T-Q^+, which then results in higher and

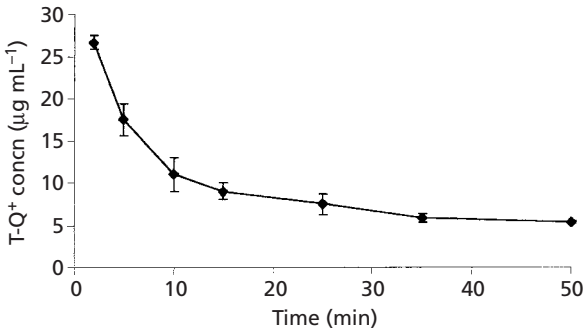

Figure 2 Concentration in blood vs time profile of T-Q^+ (**3**) after intravenous administration of 20 mg kg^{-1} of T-CDS (**4**) to dogs. Data represent average $\pm$s.d.

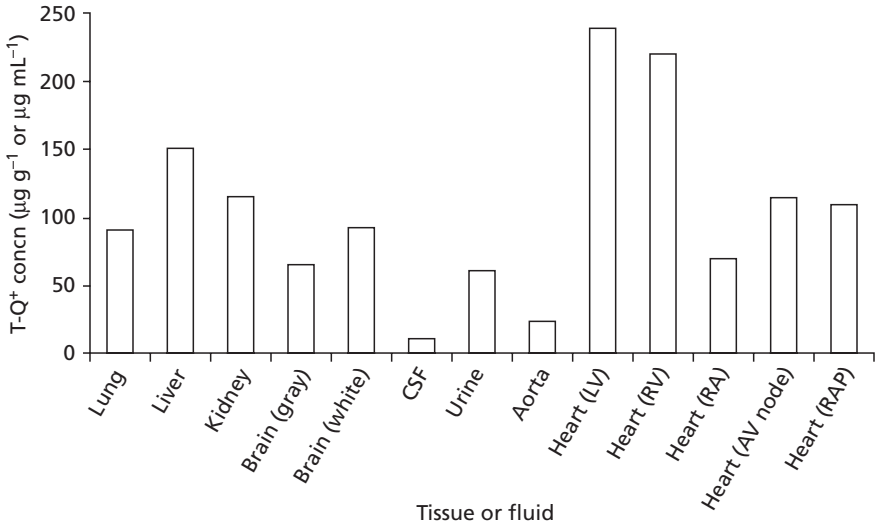

Figure 3 Concentration of T-Q^+ (**3**; μg g^{-1} or μg mL^{-1}) in different fluids and organs of dog (dose: 20 mg kg^{-1} T-CDS (**4**), i.v.) at 300 min after administration. CSF, cerebrospinal fluid; LV, left ventricle; RV, right ventricle; RA, right atrium; RAP, right appendage.

differential retention. Together these observations indicate that the rate of oxidation to T-Q^+ is different in various parts of the heart, most likely reflecting differences in blood supply, and thus effectiveness of the oxidative conversion, T-CDS $\rightarrow$T-Q^+. Consequently, this type of compound could be used for identifying ischaemic areas in the heart.

Effect on cardiac electrophysiology

The results of electrophysiology studies show that 20-mg-kg^{-1} doses of T-CDS resulted in an increase in all measured parameters, except H and HV (Figure 4), in two dogs; the effect was slightly greater in the dog given a loading bolus dose of 20 mg kg^{-1} followed by 10 mg kg^{-1} (not shown, just one dog). After the 20-mg-kg^{-1} dose, the electrophysiology parameters increased in both dogs: AH increased by 20 and 25%, SCL by 44 and 14%, SNRT by 46 and 22%, CSNRT by 75 and 57%, SACT by 18 and 51%, AERP by 6 and 48%, AVN-ERP by 31 and 48%, AVN-FRP by 20 and 38% and HPS-ERP by 15 and 38%. No change in CO was observed. It is clear that a single bolus injection has a significant effect on the sinus impulse formation and the impulse propagation. It increased both the sino-atrial conduction time and the atrioventricular conduction time. The compound had a marked effect on the atrium, AVN and HPS, which was manifested in the significant

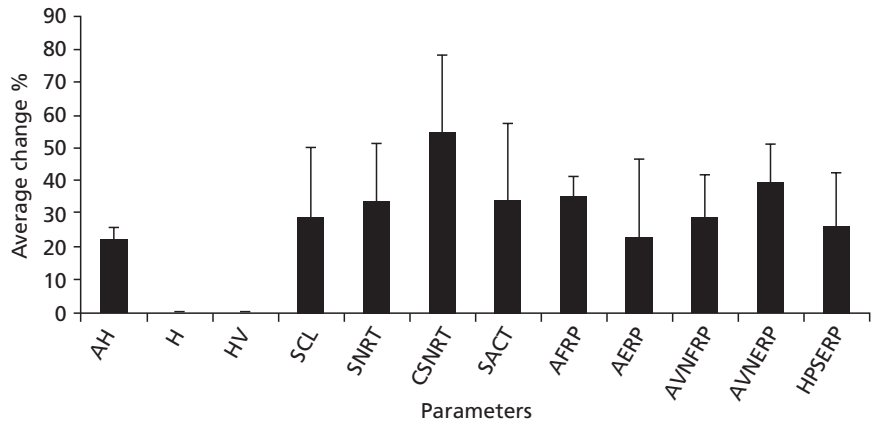

Figure 4 Cardiac electrophysiology parameters recorded in the two dogs administered 20 mg kg^{-1} of T-CDS (**4**).

increase of the refractory periods of these structures. The results suggest that T-CDS (**4**) could be an effective anti-arrhythmic agent in the different cases of supraventricular rhythm disturbances. No effect was observed on blood pressure of the treated dogs at any time of the experiment, up to 3 h.

After administration of T-Q^+ (2 mg kg^{-1} and 10 mg kg^{-1}, i.v) there were practically no changes in any of the cardiac parameters.

Conclusion

A good anti-arrhythmic must simultaneously satisfy at least two stringent criteria: the interaction with the channel must occur with the appropriate state and the binding and unbinding rates must be appropriate (Hondeghem 1995). Most of the available anti-arrhythmics have serious side-effects (Giardina & Lipka 1995), which include gastrointestinal disturbances, tinnitus, hearing loss, confusion, disturbances in vision and speech disorders, but the most critical is the possibility of exacerbation of arrhythmia (Podrid 1984). It is therefore not surprising that the currently available drugs are rather poor performers or may actually be arrhythmogenic. Therefore these drugs should be used with great caution, if at all. Accordingly, specific delivery of an anti-arrhythmic to the heart muscles may alleviate most of these side-effects. It is obvious that T-Q^+ has a marked effect on electrophysiological parameters and that T-CDS delivers T-Q^+ preferentially to heart tissue while T-Q^+ itself does not reach the heart or deep tissues in reasonable concentrations. Thus, T-CDS could be used as an anti-arrhythmic with selective delivery to the site of action, the heart muscle.

Since T-CDS $\rightarrow$ T-Q^+ conversion is an oxidative process, similar to the NADH $\rightarrow$ NAD^+ conversion, regional distribution of T-Q^+ following administration of T-CDS could visualize relative oxidative (or ischaemic) properties when radiolabelled T-CDS analogs are administered.

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