The Action of the Alkaloids from Yew (*Taxus baccata*) on the Action Potential in the *Xenopus* Medullated Axon

The poisonous nature of the leaves and berries of the yew (*Taxus baccata*) has been known for centuries yet the chemistry and physiology of the active components has been strangely neglected. The yew contains a number of alkaloids whose chemistry has been partly worked out by BAXTER et al.[1]. The main ingredients are taxicine and various esterified derivatives of it as indicated in Figure 1. This mixture is crude taxine. No recent pharmacological or physiological studies have been carried out and the only information available is that the crude taxine causes cardiac arrest in diastole[2-4].

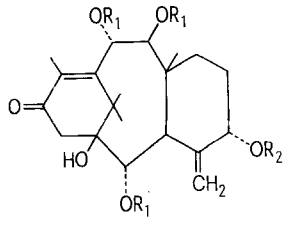

Fig. 1. Taxicine, $R_1 = R_2 =$ H. 'Taxine', $R_1 = CH_3$ or H. $R_2 =$ CO. CH_2. C. Ph. H. N $(CH_3)_2$.

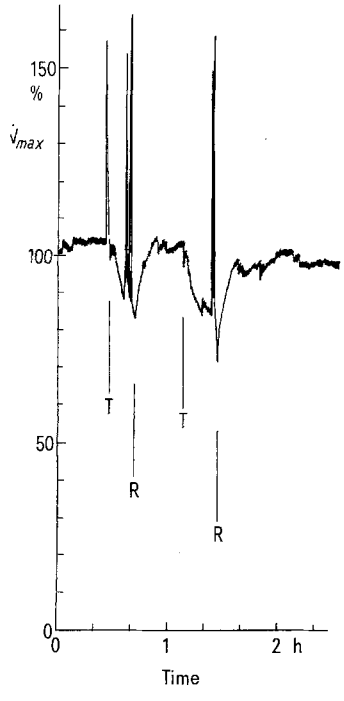

Fig. 2. Time course of crude yew taxine depression of electrical activity in the *Xenopus* single medullated nerve fibre. Ordinate, V_{max}, is percent change in peak value of the first time derivative of voltage associated with the rising phase of the action potential. Computer print out provides 300 points/h. Each point corresponds to the mean of 12 consecutive measurements taken at 1 sec intervals. Stimulus strength is adjusted so as to maintain a constant response latency. At times designated by T the fibre was exposed to 2×10^{-5} *M* crude taxine. The symbol R indicated time at which crude taxine was replaced by pure Ringer solution. Records of V_{max} in excess of 120% correspond to the maximal limiting values obtained during brief intervals of strong hyperpolarization. Continous fluid exchange was maintained throughout the experiment.

A hypothetical model of the molecular structure of the ion recognition site and the 'h' gate on the sodium channel has appeared elsewhere together with postulated mechanisms of action of tetrodotoxin (TTX) on the former and batrachotoxin (BTX), aconitine and veratradine on the latter[5]. CPK molecular models of taxicine and its derivatives suggested that taxicine itself is a structural analogue of TTX in certain respects and that di- and tri-acetyl taxicine have features in common with both BTX and aconitine. We therefore conducted experiments to determine whether the mixed alkaloids of *Taxus baccata L.* had any action on the propagated action potential in nerve.

The single axon preparation from *Xenopus* was prepared as described elsewhere[6]. The crude taxine containing taxicine and various forms of its esters was isolated from fresh yew leaves obtained from the Birmingham Botanical Gardens by a modification of the procedure described by BAXTER et al.[1]. The leaves were dried in vacuo at 40 °C for 3 days; a 100 g sample of dried leaves was extracted with 600 ml of 0.65% sulphuric acid for 4 min in a Waring blender. After standing for 3 days at room temperature, the mixture was filtered (Celite) by suction, adjusted to pH 9 by addition of aqueous ammonia and then extracted 4 times with 200-ml portions of carbon tetrachloride. Each of these extracts was shaken with the same 150-ml portion of 10% sulphuric acid, separated, and the aqueous phase was basified (cooling) with aqueous ammonia, and then extracted 6 times with 100-ml portions of ether; the combined ether extracts were dried (anhydrous magnesium sulphate) filtered, and evaporated to dryness at room temperature under reduced pressure. The crude taxine (500 mg) was a colorless amorphous powder. It was stored at −78 °C to prevent possible deterioration.

The effect of the crude taxine is shown in Figure 2. Continued exposure of the *Xenopus* single fibre to the crude taxine at a level of 2×10^{-5} *M* resulted in a gradual decline of V_{max}, the peak value of the first time derivative of voltage associated with the rising phase of the action potential. However, there appeared no change in the maximal limiting value attained by V_{max} under conditions of strong hyperpolarization.

In terms of a previous analysis[7] these findings indicate that the crude taxine has altered the parameters of sodium conductance and possibly those of potassium. Of noteworthy interest is the finding that complete recovery from depression is often apparent on removal of the crude taxine. Analysis of later and more pronounced effects awaits voltage clamp scrutiny. Thus, in conclusion we report that crude taxine from yew has an effect on the single fibre action potential, by depressing the sodium

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conductance in the absence of any effect on the electrochemical gradient. Further studies using specific constituents isolated from the crude mixture are underway.

Riassunto. La tassina cruda, ottenuta del tasso *Taxus baccata L.* ha dimostrato di ridurre l'influsso Na^+ dell'azione potenziale di singole preparazioni axon dal nervo sciatico della rana. La tassina cruda contiene una miscela di composti chimici e noi suggeriamo che la tassicina è il composto chimico responsabile per questo effetto.

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The Effect of Cold and Diazepam on the Toxicity of Fenfluramine in Mice

Fenfluramine is an amphetamine derivative and an antiobesity drug. Fenfluramine intoxications, especially in children, have often been described in literature[1–5]; 8–10 tablets (160–200 mg) alone have caused a severe intoxication in a 2.5-year-old child[5]. There exists evidence that fenfluramine has been used for hallucinogenic purposes[6], which adds to the risk of fenfluramine intoxications.

The treatment of fenfluramine overdose is primarily symptomatic, as no specific antidote exists[5]. Forced diuresis is probably of doubtful value[1]. Pyrexia, convulsions and tachycardia are characteristic of fenfluramine intoxication. Using controlled animal tests, we intend to examine whether cold treatment, diazepam and practolol have any positive effect on the treatment of fenfluramine intoxication.

Materials and methods. Male NMRI-mice used in the test were about 2 months old. The mice were bred in conventional laboratory circumstances, 9–10 of them in 1 box. The fenfluramine hydrochloride dose used in all the tests was 75 mg/kg i.p. because according to the preliminary tests it killed 0.75 of the aggregated mice. The final mortality figures were recorded in 24 h.

In cold treatment tests we used 7 groups of mice, each group consisting of 9–10 mice. The fenfluramine injection was administered at 1 min intervals to different mice of the group. The mice of the 1st group were removed to the same cold box (0 °C) instantly after the injection. The mice of the 2nd group were removed to the same cold box (0 °C) 5 min, those of the 3rd group 10 min and the mice of the 4th group 15 min after the injection. After the injection, before the removal to the cold, the animals of the group were also kept in the same box at room temperature (22 °C). 1 h after the injection the mice were brought to room temperature. The test consisted of 3 control groups. The mice of the 1st group were removed to another box at room temperature 5 min, those of the 2nd group 10 min and the mice of the 3rd group 15 min after the injection.

The effect of diazepam and practolol was examined with 9 groups of mice, each of which consisted of 9–10 mice. The fenfluramine injection was administered at 2 min intervals to different mice of the group. 1.50 min after the administration of fenfluramine the mice received i.v. either diazepam, practolol or physiological saline solution. The mice of each group were put in the same box after the injection.

Results. As soon as 5 min after the fenfluramine injection, the animals jerked and had clonic convulsions. Increased salivation and the rigidity of the limbs occurred as well. Occasional jumping attacks were developed after 7–8 min. Most animals went into a coma before death. Most lethal cases were produced 15–30 min after the injection, rarely any sooner or later. Those animals which survived were in good condition after 24 h. Increased aggressiveness was a frequent symptom.

Cold treatment decreased or abolished the hyperactivity and the convulsions caused by fenfluramine. The effect of cold treatment on the toxicity of fenfluramine has been described in Table I. It can be observed that when the

Table I. The effect of cold treatment on mortality caused by fenfluramine (75 mg/kg i.p.) in mice

Removed to cold (0 °C) after	*n*	Mortality (%)	Significance (p)
Control	29	83	
0 min	10	0	< 0.001
5 min	10	0	< 0.001
10 min	9	11	< 0.001
15 min	10	40	< 0.05

Table II. The effect of diazepam and practolol on mortality caused by fenfluramine (75 mg/kg i.p.) in mice

Post-treatment after fenfluramine	*n*	Mortality (%)	Significance (p)
Saline	28	71	
Diazepam (2 mg/kg)	10	0	< 0.001
Diazepam (0.5 mg/kg)	10	0	< 0.001
Diazepam (0.2 mg/kg)	19	21	< 0.01
Practolol (2 mg/kg)	10	60	n.s.
Practolol (5 mg/kg)	9	11	< 0.01

Diazepam, practolol and physiological saline solution were administered into the tail vein 1.5 min after the fenfluramine injection.

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