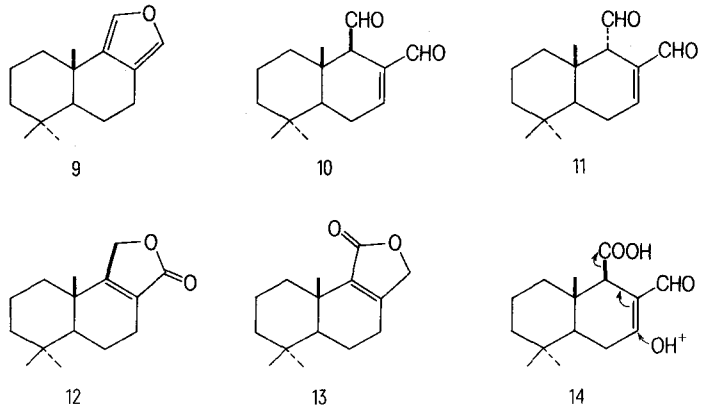

9 10 11

12 13 14

11 and **12,** and the biogenetic consideration (hydroxylation-decarboxylation) indicated in the formula **14,** show that the structure of polygonal is most favourably represented as the formula **1.** Polygonal has recently been afforded during the course of the synthesis of warburganal[4] and the spectral data of the natural polygonal were completely identical with those of the synthetic one[5].

Iso-Drimeninol (**5**), $C_{15}H_{24}O_2$ (M^+ 236), $[\alpha]_D$ −37° (c, 1.5), indicated the presence of a hydroxyl group (3400 cm^{-1}), 3 tertiary methyl groups (0.80, 0.86, 0.93 ppm, s, 9H), an isolated methylene group in an asymetrical environment attached to oxygen atom (4.15 and 4.50 ppm, d, J = 11 Hz), a carbinyl proton (5.26 ppm, d, J = 4 Hz) and a vinylic proton (5.50 ppm, bs). Reduction of **5** with $LiAlH_4$ gave a diol, m.p. 72–73 °C $[\alpha]_D$ −6.5° (c, 0.9), whose spectral data and physical constants were in accordance with drimane diol (**8**)[2,6,7], indicating that **5** possessed drimane skeleton with the same absolute configuration as polygodial (**10**).

Acetylation of **5** with acetic anhydride in pyridine afforded a monoacetate (**6**) ($[\alpha]_D$ −42.1° (c, 3.8); $C_{17}H_{26}O_3$ (M^+ 278); 1742, 1230 cm^{-1}; 2.02 ppm (s, 3H), together with a small amount of β,β-disubstituted furano compound (**9**) [$C_{15}H_{22}O$ (M^+ 218); 7.10 ppm (bs, 2H)][7,8]. The mild oxidation of **5** with Collin's reagent gave isodrimenin (**13**)[6]. The above chemical transformation showed that **5** was drimane type sesquiterpene hemiacetal with a hydroxyl group at C-11.

In fact, the IR-spectrum and the chemical properties of **5** were identical to those of drimeninol (**7**) which has been isolated from the liverwort[7]; however, their NMR-spectra and chromatographic behaviour was slightly different, conclusively establishing the structure **5** for isodrimeninol.

Polygonal (**1**) is the second pungent substance found in *P. hydropiper,* but its pungency is fairly weak in comparison with that of polygodial (**10**). Polygodial and polygonal completely inhibited the germination of the rice husk at ca. 100 and 500 ppm, respectively.

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Post mortem changes in adenylate cyclase activity in rat brain striatum

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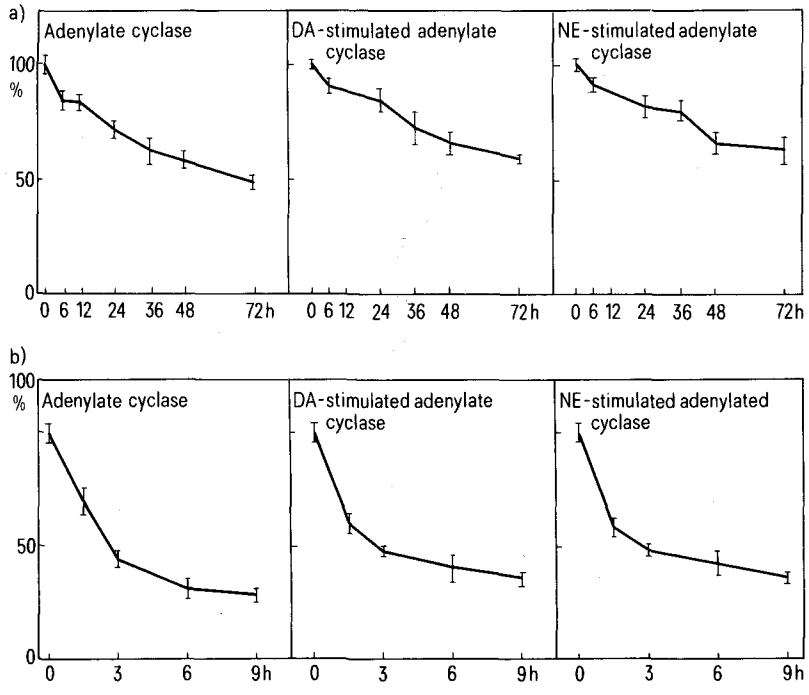
Summary. Adenylate cyclase activity in rat striatum decreased post mortem. Half-lives were about 2.7 h at 22 °C, 72 h at 4 °C. Differences in stability after death of adenylate cyclase in human brain and rat striatum, and possible heterogenity of the enzyme, are briefly discussed.

There is strong evidence that cyclic AMP plays an important role in certain types of synaptic transmission, e.g. in catecholamine-mediated transmission. The properties of adenylate cyclase, the enzyme synthesizing cyclic AMP from ATP, have been investigated in brain tissue from, for example, rabbit, mouse, rat and monkey. There have been only a few and conflicting publications concerning adenylate cyclase in human brain: it was detected in slices of fresh surgery material, and its activity could be stimulated by various biogenic amines[2,3]. Activity of the enzyme was also detected in homogenates from various areas of human brain obtained 10 h after death; it was, however, not sensitive to catecholamines[4]. In contrast, Clément-Cormier et al.[5] could detect and stimulate the enzyme in homogenates of human striatum obtained 4 h after death.

Since the conflicting data could be the result of post mortem changes and since autopsy material is more readily available than surgical samples for the study of the human



Time course of adenylate cyclase activity during post mortem storage of brains at 4 °C (a) or 22 °C (b) as measured in homogenates without and with added 0.1 mM dopamine (DA) or 1 mM L-norepinephrine (NE), respectively. Initial adenylate cyclase activities (pmoles cAMP/mg wet weight × 5 min) were: Basal activity = 70.1 ± 2.8 (SEM); dopamine-stimulated activity = 143.4 ± 1.9; L-norepinephrine-stimulated activity = 147.5 ± 3.8.

enzyme, it appeared desirable to study the stability of the enzyme and its sensitivity to biogenic amines after death. Rat striatum was chosen as a model.

Male albino Wistar rats (160–180 g) were decapitated (between 8.30 and 9.30 h), and the heads maintained at room temperature (about 22 °C) or 4 °C, respectively, for different periods. Thereafter, the braines were rapidly removed, the striata isolated and gently homogenized in 100 vol. (w/v) of ice-cold 2 mM Tris (hydroxymethyl)-aminomethane-maleate buffer – 2 mM EGTA-NaOH (pH 7.4) using a glass homogenizer with a Teflon pestle. Incubations were carried out in a total volume of 200 µl containing (in mmoles/l): tris (hydroxylmethyl)-aminomethane-maleate, 80.25; $MgSO_4$, 8.0; theophylline, 8.0; EGTA, 0.25; ATP, disodium salt, 1.5; 30 µl of tissue homogenate plus catecholamines as indicated in the figure. The reaction was started by the addition of ATP and stopped, after 5 min incubation in a shaking water bath at 37 °C, by placing the assay tubes in a boiling water bath for 2 min. The amount of cyclic AMP formed was measured in duplicate by the protein binding method of Gilman[6] using 50 µl-aliquots from each assay tube.

Storage of the heads at 4 °C resulted in a slow loss of both dopamine or L-norepinephrine-stimulated and unstimulated adenylate cyclase activities. 72 h post mortem, about 50% of the initial activities were still present. The basal activity and the total activities measured with either dopamine or L-norepinephrine decreased approximately similarly.

Storage of the heads at room temperature (22 °C) resulted in a more rapid loss of both catecholamine-stimulated and unstimulated adenylate cyclase activities. Half-life of the enzyme was about 2.7 h. After 9 h, activities had fallen to about one third. Half-lives of the unstimulated and catecholamine-stimulated activities seemed equal at 22 °C. During storage of the homogenates at – 15 °C for several months, both catecholamine-stimulated and unstimulated adenylate cyclase activities decreased only slightly.

A number of enzymes involved in catecholaminergic transmission were rather stable post mortem and could be assayed in homogenates from both rat and human brains: Dopamine-β-hydroxylase, monoamine-oxidase and catechol-O-methyltransferase[7]. Dopa-decarboxylase, on the other hand, was rather stable for many hours of storage in rat brain[7,8], whereas it could not be detected in human brain even only 5 h after death[7]. A similar difference between human and rat striatum was observed for unstimulated and catecholamine-stimulated adenylate cyclase (unpublished results). Possible reasons for this difference, which seem to rather discourage investigations of this enzyme in autopsy material, could be 1. different conditions of human deaths, causing more or less damage to adenylate cyclase activities already inside the brains; 2. a different and less stable structure of human adenylate cyclase; 3. an endogenous inhibitor masking the stimulation by catecholamines – one or more – in human brain; 4. assay conditions optimized for the rat enzyme might be detrimental to the human enzyme.

Adenylate cyclase activities in striatal tissue might be heterogenous. Small differences observed between half-lives of total activities in the presence and absence of catecholamines might indicate heterogeneity. Further investigations were concerned with this question of heterogeneity (unpublished results): K_m-values of adenylate cyclase for ATP, being in the order of $2.4 \pm 0.8 \times 10^{-4}$ M, showed no significant differences between the unstimulated and the dopamine-stimulated enzyme.

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