clonazepam's anticonvulsant activity was potentiated by all the drugs tried. In any case, it has not been possible at present to find 'model animals' in which all kind of human epilepsy can be reproduced[14]. We believe that the different behaviour of all the drugs tried with clonazepam could be explained by the different mechanisms of action each of them has[13]. Barbiturates, phenurone and diphenylhydantoin have a common mode of action, although the first ones have a more sedative and depressive activity of the CNS than diphenylhydantoin. We have to point out that the doses of barbiturates and phenurone required to protect from the effect of maximal electroshock are higher than the doses required to protect from the effects of cardiazol; on the other hand, the ED_{50} of diphenylhydantoin required to protect against cardiazol convulsions is higher than the dose required to protect from the electroshock convulsions. This induces us to think that diphenylhydantoin posses certain peculiarities with respect to barbiturates which would explain why although they have similar antiepileptic activity, they behave dif ferently with respect to the anticonvulsant activity of clonazepam in convulsion–induced by cardiazol.

Therefore it can be concluded that: a) The cardiazol technique we have used induces particularly epilepsy minor crisis. This agrees with the results obtained by Toman[8]. b) The maximal electroshock technique is not specific for studying the effect of several associations of antiepileptic drugs. c) We believe that, although barbiturates and diphenylhydantoin have been grouped together as having similar mechanism of action and similar therapeutic indications, they have anticonvulsive peculiarities which can clearly be observed with the experimental technique used.

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The effect of verapamil and dibutyryl cAMP on the spontaneous activity of the sinus node

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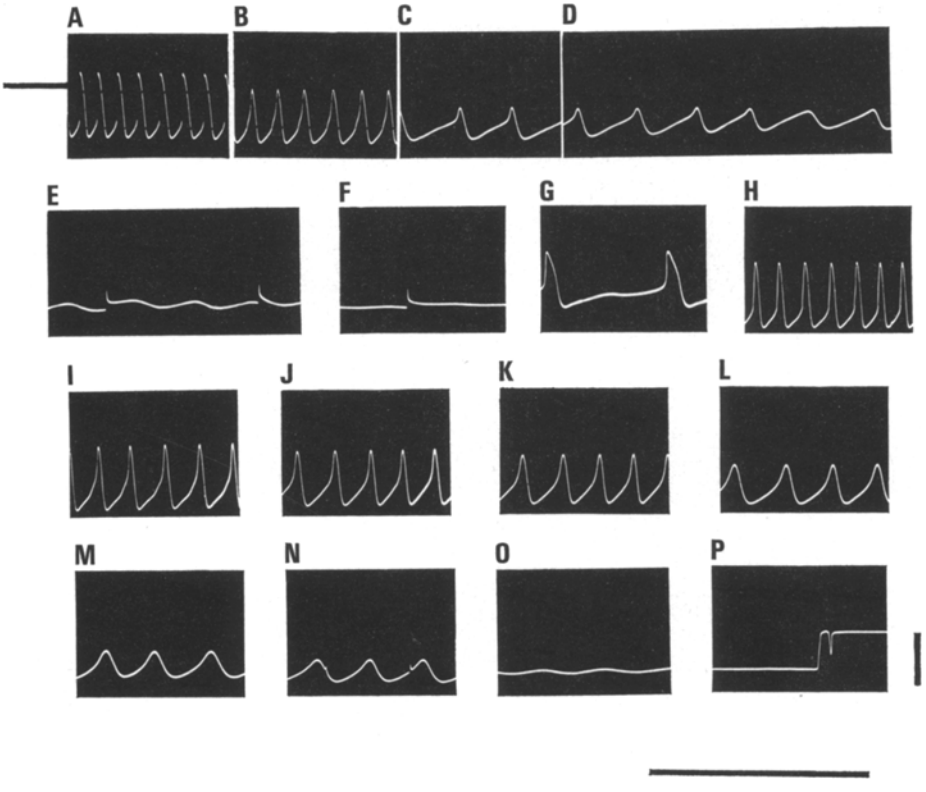
Summary. Verapamil stops the electrical activity of the sinus node cells. In the presence of verapamil, dibutyryl cAMP brings about a recovery of the spontaneous activity, whereas noradrenaline is ineffective.

Verapamil was initially thought to be a specific Ca-antagonist for smooth and cardiac muscle[1–4]. It has now been recognized that only (–)-verapamil exerts an inhibitory action on the slow Ca-channel, whereas its (+)-isomer interfers with the fast Na-channel[5, 6]. Thus racemic and (–)-verapamil can suppress the slow component of the action potential in myocardial cells[6–9]. The aim of present study was to investigate how verapamil acts on the electrical activity of the sinus node cell in the pacemaker region, and to find out if noradrenaline and dibutyryl cAMP (db cAMP) could counteract the verapamil effects. In these experiments, racemic verapamil was employed in a concentration that left the fast channel practically unaffected[6].

Methods. The experiments were performed on 24 isolated sinus nodes of the rabbit heart. Each preparation was



Effect of verapamil and db cAMP on the sinus node cell. *A* control, *B–F* action of verapamil, *B* 3 min, *C* 12 min, *D* 12 min 5 sec, *E* 15 min, *F* 18 min of perfusion; *G–H* db cAMP added to perfusion fluid containing verpamil, *G* 8 min, *H* 10 min of perfusion; *I–P* rinsing with Tyrode fluid containing verapamil, *I* 10 min, *J* 20 min, *K* 25 min, *L* 40 min, *M* 48 min, *N* 60 min, *O* 73 min, *P* 75 min of perfusion. In *E*, *F* and *N* the cell was stimulated. Calibration: voltage 50 mV, time 5 sec.

perfused with oxygenated and warmed (35 °C) Tyrode solution. Electrical activity was recovered by means of conventional glass microelectrodes. Each recording microelectrode was also used for intracellular stimulation (current 1–2 μA, duration 3 msec, frequency 0.2–1 Hz) of the cell[10]. After control records, the preparation was perfused with Tyrode solution containing 5×10^{-7} M/l of (±)-verapamil until complete cessation of spontaneous activity ensued. About 5 min after arrest, the preparation was perfused with Tyrode solution containing verapamil and 10^{-5} m/l of noradrenaline bitartarate or 2×10^{-3} M/l of db cAMP.

Results and discussion. The effects of verapamil and db cAMP are shown in the figure. Verapamil reduced the spontaneous rate, decreased the slope of the slow depolarization and diminished the amplitude of the action potential; 12–40 min from the onset of verapamil administration, the action potentials disappears and the records showed only slow depolarization. Within this period, the cells became inexcitable both for depolarizing and for hyperpolarizing pulses (figure, E, F), although in the control conditions, intracellular stimulation during slow depolarization and at the end of repolarization was always effective. In a further 3–8 min, the amplitude and rate of the slow depolarization progressively decreased and finally a stable membrane potential of –45 to –50 mV was recorded.

Addition of noradrenaline was ineffective, whereas db cAMP caused the spontaneous activity to return within 5–20 min. This activity remained as long as the nucleotide was present. Washing out the preparation with pure Tyrode solution, or Tyrode solution containing verapamil, again caused an arrest within 25–75 min. The above results confirm other investigations which indicate that in the cardiac pacemaker slow depolarization as well as the action potential is mainly dependent on the calcium influx[11–13]. Disappearance of the action potential and the complete inexcitability of the cells arrested by verapamil could also support the view that, in the nodal action potential, only the slow current component is present[14].

However, the effect of the db cAMP in the presence of verapamil remains unclear. It is generally accepted that verapamil inhibits calcium influx acting directly on the slow channel[3, 4, 15]. This may explain the lack of action of noradrenaline which, as its actions, increases the inward Ca-current[16, 17]. However, it does not explain why db cAMP reactivates spontaneous activity blocked by verapamil which only slightly, if at all, affects the cAMP content in the heart and does not counteract the adrenergic stimulation of adenylate cyclase[15, 18]. The last finding makes an unspecific action of db cAMP unlikely, because otherwise elevated level of cAMP due to noradrenaline would be also effective.

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Spectral changes resulting from the interaction of some N-alkyl nitrosamines and rat liver microsomes[1]

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Summary. Dimethyl (DMN) and diethyl nitrosamine(DEN) do not give characteristic spectral changes upon interaction with rat liver microsomes, while dipropyl (DPN) and dibutyl (DBN) nitrosamine cause type I spectral changes. The spectral binding constant is 100 mM for DPN and 1.17 mM for DBN. The maximal spectral change is 3.2×10^6 and 1.0×10^6 absorbance units per milligram protein for DPN and DBN respectively.

Many compounds known to be substrates for the hepatic microsomal mixed function oxygenase have been shown to interact with oxidized cytochrome P450 (P450). Remmer et al.[2] using difference spectroscopy showed 2 distinct types of spectral shifts which they later called type I and II. The type I shift was typified by compounds such as aminopyrine, hexobarbital and many other chemicals and was characterized by a trough of about 420 nm and a peak at 385 nm. Compounds such as aniline, pyridine and others gave type II shift, characterized by a peak at about 430 nm and a trough at 395 nm. Type I spectra are generally considered to be caused by nonspecific interactions with a lipophilic site other than the

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