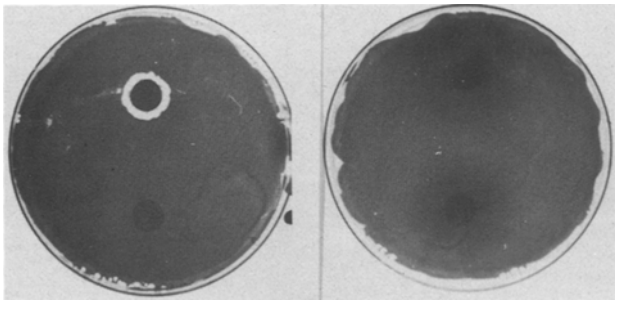
respiratory tracts. Even more serious, a systemic infection may affect the circulatory, respiratory, digestive, urinary, or central nervous systems, as well as other organs such as liver, spleen, or pancreas. The use of *C. albicans* should therefore not be undertaken casually by anyone, and it should be strongly discouraged in the absence of overriding justification, a fact which was not mentioned by the above workers in this field.

We wish to demonstrate that *C. utilis,* a common food yeast, is an excellent substitute for *C. albicans.* As shown in the pictures (figure) a clear positive test was obtained with a classical phototoxic compound such as 8-methoxypsoralen. A positive response was also obtained with α-terthienyl. The phototoxicity of the latter had been discovered through a test performed with *C. albicans*[5], and it would certainly not have been missed had *C. utilis* been used instead. Typical photodynamic compounds such as rose Bengal (figure), hypericin, and methylene blue were found to give a negative test for phototoxicity with *C. utilis.* Negative results had also been obtained for the first 2 compounds with *C. albicans,* but the 3rd had given variable results with this organism.

In conclusion, *C. utilis* appears to be in every respect as good as *C. albicans* for the phototoxicity tests, with the added advantage of not being pathogenic. Therefore, there does not seem to be any justification for using the latter organism in investigating the phototoxicity of either chemicals or plant samples.

Experimental. Candida utilis was grown on a TGY medium, consisting of 1% tryptone, 2% glucose, and 0.5% yeast extract. TGY agar plates were inoculated evenly with *C. utilis* from a 48-h slant, and the chemical to be examined was applied to a piece of Whatmann No. 1 filter paper, about 1 cm in diameter, which had been previously sterilized with 95% ethanol. The paper was placed on the plate, which was irradiated from a distance of 10 cm with a 19.5-W UVL-22 'Black-Ray' UV-lamp, with an emission at 320–390 nm. A blank similarly prepared was kept in the dark for the length of the irradiation (12–24 h). A positive response could be observed with 10^{-7} g of 8-methoxypsoralen, or 10^{-6} g of α-terthienyl.



Phototoxicity test with *C. utilis.* Left: 8-methoxypsoralen, right: rose Bengal. In each case the lower half of the plate was shielded from the light with an aluminium foil.

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The effects of β-sympathomimetic amines and phosphodiesterase inhibitors on electrophysiological parameters in Purkinje fibres[1]

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Summary. Dose-dependent responses to L-isoprenaline of spontaneous activity and plateau height in Purkinje fibres can be mimicked closely by the PDE-inhibitor IBMX. Simultaneous applications of sympathomimetic amines and IBMX result in potentiated responses. The results support the hypothesis that cyclic-AMP is the common mediator of positive chronotropic and inotropic effects induced by β-sympathomimetic amines.

Catecholamines act in a characteristic way upon heart preparations: the effect on the pacemaker potassium current (ik_2) starts at relatively low concentrations and can be described by a 1:1 binding curve[3]. The increase in slow inward current (i_{si}) becomes visible at higher concentrations and the dose response curve suggests a 2:1 relation[4]. Both effects have been proposed to be mediated by the intracellular 'second messenger', cyclic-AMP (adenosine 3′,5′-monophosphate)[5]. In spontaneously active fibres the attempt to correlate the catecholamine effects with an increase in the intracellular level of cyclic-AMP has only been partly successful[6,7]. High doses of catecholamines produce a distinct elevation of adenyl cyclase activity, whereas with low but still efficacious doses, which mainly effect the pacemaker current, a significant increase is not detected. This observation might be explained either by assuming that part of the positive chronotropic effect is not mediated by cyclic-AMP, or that the change in the functionally relevant fraction of cyclic-AMP is too small to be detected, e.g. because a large background level of cyclic-AMP might be present. In order to avoid the difficulties of cyclic-AMP determinations the following approach seems to be applicable: if, as suggested, both effects are mediated by cyclic-AMP, similar characteristic dose-response relations for both effects should be obtained by an inhibition of the phosphodiesterase (PDE) which converts cyclic-AMP into inactive 5′-AMP. In addition an inhibition of the PDE together with a simultaneous stimulation of the β-receptor

should result in a potentiation of both responses[5]. Here we present experiments with a highly potent PDE-inhibitor in order to test the predictions described.

Methods. All experiments were performed on isolated calf or sheep Purkinje fibres. Intracellular action potentials were recorded in the usual way with KCl-filled microelectrodes. Spontaneous activity commenced as a rule after superfusing the preparation with a Tyrode solution of the following composition (mM): NaCl (137), KCl (2.7), $MgCl_2$ (1.05), $NaHCO_3$ (11.9), NaH_2PO_4 (0.24), $CaCl_2$ (1.8), and glucose (5.5) (saturated with 95% O_2 and 5% CO_2, temperature 35 ± 0.2 °C). Ca-mediated action potentials were elicited by field stimulation after increasing KCl to 21.6 mM in the otherwise unaltered Tyrode solution. We evaluated 1. the frequency of spontaneous activity, 2. the slope of diastolic depolarization and 3. the maximum plateau height. Alterations of these parameters were taken as an indirect measure of drug action on pacemaker potassium current (1. and 2.) and slow inward current (3.), respectively. The applicability of indirect parameters in this context has been discussed in detail elsewhere[8]. As sympathomimetic amines, L-isoprenaline and 1-isopropylamino-3(4-hydroxyphenoxy)-propran-2-ol (L-IHP)[9] were applied and as a PDE-inhibitor, 3-isobutyl-1-methylxanthin (IBMX) was used.

Results and discussion. In figure 1, A the effects of L-isoprenaline in the steady state upon spontaneously beating calf Purkinje fibres are shown. At a relatively low concentration (10^{-8} M) the drug caused an increase in the frequen-

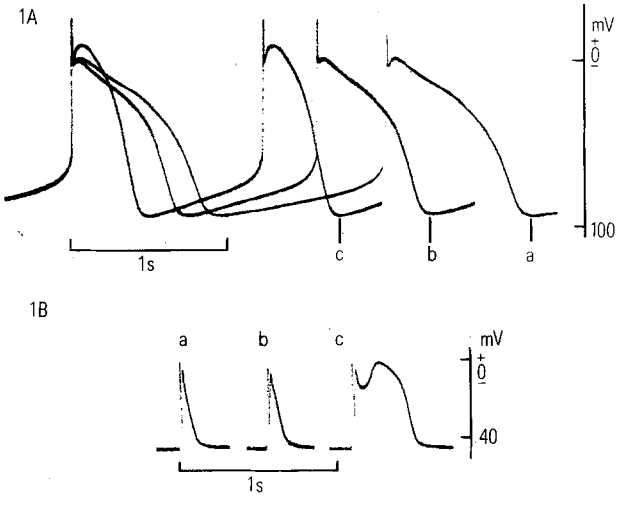

Fig. 1. A, action potentials in spontaneously beating calf Purkinje fibres after 10 min equilibration in Tyrode solution a) without and b) with 10^{-8} M or c) 10^{-6} M L-isoprenaline. B, membrane potential response to a supramaximal stimulus in 21.6 mM K^+ Tyrode solution, containing a) no drugs and b) 10^{-8} M or c) 10^{-6} M L-isoprenaline.

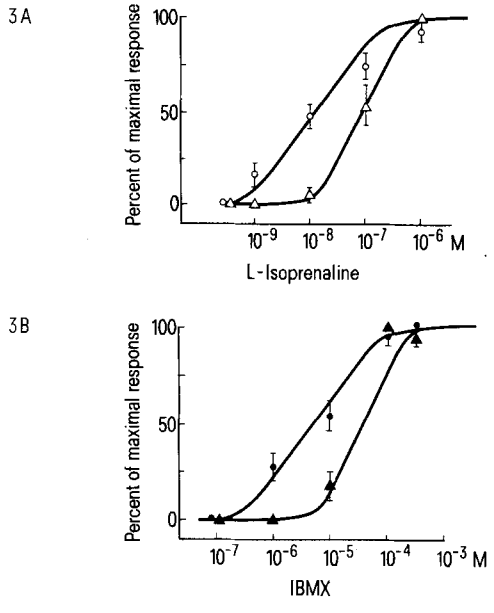

Fig. 3. Dose response curves for the action of A, L-isoprenaline (n=7, ± s.e.m.) and B, 3-isobutyl-1-methylxanthin (IBMX) (n=6) upon alterations in the slope of the diastolic depolarization (○, ●), and the height of the plateau phase (△, ▲). The data were fitted by eye to a 1:1 and 2:1 binding curve, respectively.

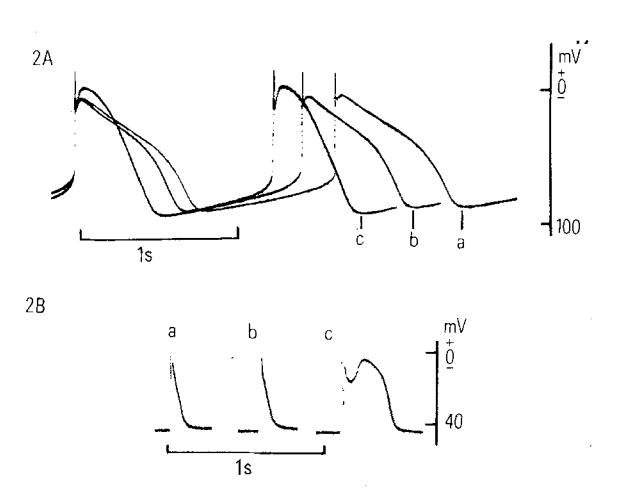

Fig. 2. A, action potentials in spontaneously beating calf Purkinje fibers after 10 min equilibration in Tyrode solution a) without and b) with 10^{-6} M or c) 10^{-4} M 3-isobutyl-1-methylxanthin (IBMX). B, membrane potential response to a supramaximal stimulus in 21.6 mM K^+ Tyrode solution containing a) no drugs, b) 10^{-6} M or c) 10^{-4} M IBMX.

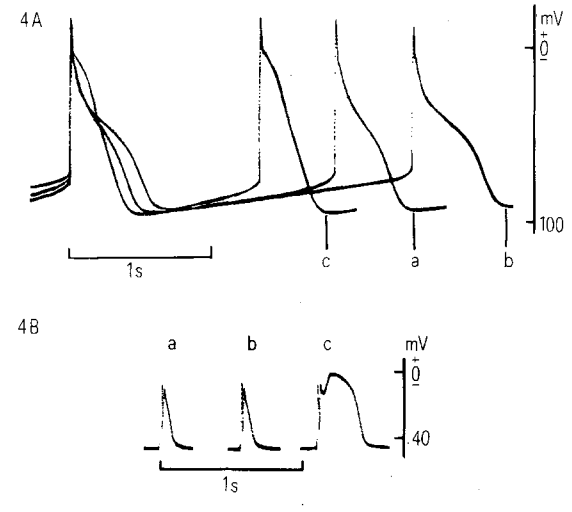

Fig. 4. A, action potentials in a spontaneously active sheep Purkinje fiber in Tyrode solution containing a) 5×10^{-6} M IBMX, b) 10^{-9} M L-isoprenaline or c) both. B, membrane potential response to a supramaximal stimulus in 21.6 mM K^+ Tyrode solution containing a) 10^{-5} M IBMX, b) 10^{-8} L-isoprenaline or c) both drugs, in a calf Purkinje fibre.

cy and slope of diastolic depolarization. A higher concentration (10^{-6} M) induced an elevation of the plateau level, in addition to a further increase in frequency and diastolic depolarization. The first increase in frequency and slope of diastolic depolarization occurred at 10^{-9} M and the maximum was reached near 10^{-6} M leading to an increase in frequency of 0.25 ± 0.05 Hz (n = 7, s.e.m.) and in the slope of diastolic depolarization by 10.1 ± 1.7 mV/sec. The first increase in plateau height was observed near 3×10^{-8} M and the maximal effect at 10^{-6} M (18.1 ± 2.6 mV). The characteristic difference in the dose-response relationships was confirmed, in addition, by the observation that Ca-mediated action potentials could only be elicited at high concentrations of L-isoprenaline (10^{-7}–10^{-6} M, figure 1, B).

A similar dissociation of the dose-dependent behaviour of spontaneous activity and plateau height was found after the application of the PDE-inhibitor IBMX (figure 2, A). The threshold for the increase in frequency and slope of diastolic depolarization was near 5×10^{-7} M and the maximum was reached at 10^{-4} M, inducing a maximum increase of 0.28 ± 0.08 Hz (n = 6) and 8.8 ± 1.9 mV/sec, respectively. The threshold for the elevation of the plateau level was reached at 10^{-5} M and the maximum (12.0 ± 1.7 mV) near 10^{-4} M. Analogously with the L-isoprenaline action, Ca-mediated action potentials could be elicited only in the high IBMX concentration range (figure 2, B). The same effects were observed in a Tyrode solution containing 2.5×10^{-7} g/ml propranolol. The β-blocker propranolol was used to suppress the effects of noradrenaline which might be released by IBMX from sympathetic nerve endings.

The following similarities in the action of L-isoprenaline and IBMX could be demonstrated: with both drugs the threshold for the increase in plateau height was reached at a 30–50 times higher concentration than the threshold for the increase in spontaneous activity. The dose-response curves for the increase in slope of diastolic depolarization could roughly be fitted by a 1:1 binding curve, and for the increase in plateau height by a 2:1 binding curve. These relationships are represented in the dose-response curves in figure 3, A, B. Similar curves have been described for β-sympathomimetic actions on the pacemaker potassium current[3] and calcium inward current[4] respectively.

The observation that over the whole concentration range IBMX closely mimicked the effects of L-isoprenaline by increasing the spontaneous activity and plateau height supports the hypothesis that cyclic-AMP mediates the entire catecholamine action. The simulation was not possible under the action of other PDE-inhibitors (e.g. caffeine and theophylline). This difference indicates that the action of IBMX in inhibiting the PDE is possibly not obscured by additional effects. IBMX has been demonstrated to be 20–50 times more potent than the other PDE-inhibitors mentioned[10]. Its high potency seems to be an essential factor in mimicking the catecholamine response. This is indicated by a similar successful simulation of the positive inotropic effect in guinea-pig papillary muscle[11].

Additional support in favour of cyclic-AMP as the mediator in the whole effective concentration range of L-isoprenaline can be derived from potentiated responses after simultaneous applications of L-isoprenaline and IBMX. The concentrations used had no effect on the plateau phase when applied separately, but led to a pronounced increase in frequency and plateau height when added simultaneously (figure 4, A). Under similar conditions Ca-mediated action potentials could be elicited (figure 4, B). The resultant effects were larger than those calculated by addition of the 2 single dose responses.

Similar results were obtained when a maximal chronotropic dose of L-IHP (10^{-5} M), a new partial agonistic β-sympathomimetic amine, which even in maximal effective concentrations neither increased the plateau level nor elicited Ca-mediated action potentials[8], was added to a low concentration of IBMX (10^{-5} M). Under these conditions an elevation of the plateau phase and an overadditive increase in spontaneous activity was observed and under comparable conditions Ca-mediated action potentials were observed in a high-K Tyrode solution. Such potentiated responses had not been possible during simultaneous applications of L-IHP and L-isoprenaline[8].

Potentiated responses after the addition of catecholamines to a PDE-inhibitor treated preparation have been stated as one criterium for a cyclic-AMP mediated response[5]. For this reason the results indicate that cyclic-AMP is the probable mediator for the increase in spontaneous activity and in plateau elevation in Purkinje fibres caused by catecholamines.

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