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COMMUNICATIONS

Photoaffinity labelling of dopamine receptors in molluscan smooth muscle

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Relaxation of catch contraction of the anterior byssus retractor muscle of the sea mussel *Mytilus edulis* L. by dopamine is mediated through a dopamine receptor but not through adrenoceptors (Takayanagi et al 1981). Photoaffinity labelling is a technique widely used in biochemical in vitro studies to test an interaction between a ligand and its binding site. Therefore, we tried photoaffinity labelling of the dopamine receptor in order to study the dopamine receptor in the anterior byssus retractor muscle of *M. edulis*.

Sea mussels, collected from the east coast of Tokyo Bay were stored in aerated seawater (NaCl 456, KCl 11, $CaCl_2$ $2H_2O$ 11, $MgCl_2$ $6H_2O$ 48 nM and Tris-HCl 25 mM; pH 7·8 to 8·0) at 10 °C and used within a week of collection. Muscle bundles (about 1 mm in diameter) were dissected from the anterior byssus retractor muscle and suspended in a 10 ml organ bath filled with artificial seawater bubbled with air and kept at 24 to 25 °C. Responses to drugs were recorded isotonically under a tension of 0·2 g. After the muscle had been exposed to acetylcholine (10^{-4} M) for 2 min to induce catch contraction and washed with artificial seawater for 5 min, dopamine was applied. Relaxations following a 10 min exposure to various doses of dopamine were estimated. The response to 3×10^{-7} M dopamine was considered as the maximum response to obtain dose-response curves (Takayanagi et al 1981).

To irradiate the muscle, a Toshiba lamp FL-20E (wavelength: 270 to 350 nm) was used as a light source. The muscle, immersed in artificial seawater containing dopamine (10^{-4} M), was irradiated (1 cm from the lamp) for 25 min and then washed with artificial seawater for 60 min (Takayanagi et al 1976).

After the muscle was irradiated in the presence of dopamine (10^{-4} M) for 25 min and washed for 60 min, the dose-response curve of dopamine was shifted in a parallel way towards doses about 8 times higher (Fig. 1). This inhibition of dopamine-induced responses continued for at least 2 h. The dose-response curve for dopamine was unaffected when the muscle was incubated with both dopamine (10^{-4} M) and haloperidol (10^{-4} M) for 25 min under the irradiation conditions (Fig. 1). However, the inhibitory action of dopamine was unaffected when the muscle was irradiated in the absence of dopamine and washed for 60 min, suggesting that 20 min irradiation did not influence mechanisms for relaxation of this smooth muscle by dopamine. Furthermore, when the muscle was incubated with dopamine (10^{-4} M) or haloperidol (10^{-4} M) for 25 min and washed with artificial sea water, the dose-response curve for dopamine was not influenced. When promethazine (10^{-4} M), an antihistamine drug found to have no antidopaminergic action in this muscle (Yoshida et al 1981), was used instead of haloperidol (10^{-4} M), the dose response curve for dopamine was shifted after irradiation (data not shown). These results indicate the possibility that dopamine is photolysed to a reactive compound which reacts irreversibly with the dopamine receptor.

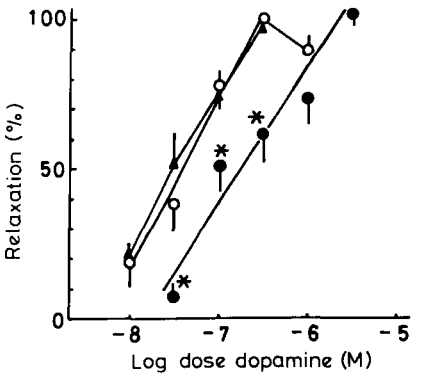

FIG. 1. Effects of irradiation on dose-response curves for dopamine in the presence of dopamine alone and of both dopamine and haloperidol. Ordinate: relaxation (%), abscissa: log dose (M) of dopamine, ○: control response to dopamine, ●: after irradiation in the presence of dopamine (10^{-4} M) and ▲: after irradiation in the presence of both dopamine (10^{-4} M) and haloperidol (10^{-4} M), a protector of the dopamine receptor. Each value is presented as a mean with s.e. of 5 experiments. *: significant difference from the corresponding control value at $P < 0.01$.

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It is known that catecholamines are photolysed (Takayanagi et al 1976). The principle of this study is based on the fact that a radical generated from dopamine makes a covalent bond with an amino acid residue in the binding site. Photoinactivation was eliminated in the presence of haloperidol, a protector of the dopamine receptor. It is well known that the dose-response curve of the agonist for which there are spare receptors is shifted by an irreversible agent (Nickerson 1956). There is a considerable shift in the dose-response curve for dopamine by photoinactivation of the dopamine receptor in this study. This possibly points to a certain receptor reserve for dopamine in this muscle. Furthermore, the fraction (q) of an amount of the dopamine receptor remaining in active form after photoactivation of the receptor and the dissociation constant (K) of the dopamine-receptor complex were estimated. The data in Fig. 1 were analysed (Furchgott 1966) by plotting reciprocals of doses of dopamine against reciprocals of equiactive doses after photoinactivation of the dopamine receptor. From the straight line best fitting the points on the reciprocal plot, the q- and K-values were calculated. The estimated q-value was 0·34. This indicates that the amount of receptor in active form is reduced after irradiation, though the maximum response to dopamine is still obtained. And the estimated K-value was $1·1 \times 10^{-6}$ M. This value coincided with the K_i-value for dopamine against the specific binding of [^{3}H]haloperidol to calf brain membranes (Burt et al 1976) or of [^{3}H]domperidone to mouse striatal membranes (Baudry et al 1979). This muscle is probably suitable for studies on the dopamine receptor. We thank Miss Y. Nakahara for skilful technical assistance.

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Pyridoxal complexes as potential chelating agents for oral therapy in transfusional iron overload

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Iron chelation therapy for patients maintained on a regular transfusion regime is at present best carried out by means of daily infusions of desferrioxamine (Hussain et al 1977; Pippard et al 1978) but this is onerous for the patient and has social and economic disadvantages. Many recent attempts to provide more effective drugs for iron chelation have been summarized by Jacobs (1979) and increasing attention is now being paid to the possibility of oral iron chelation therapy. Hoy et al (1979) showed that when isonicotinic acid hydrazide (INH) and pyridoxal are mixed in equimolar amounts a hydrazone is formed which chelates iron, and oral administration of this compound to rats results in an eightfold increase in faecal iron excretion. It is effective on repeated administration (Cikrt et al 1980), the main route of iron excretion being through the bile. Long term studies in the rat have not been successful in reducing the iron load of test animals and this appears to be related both to their high dietary iron content and instability of the hydrazone. Its effective shelf life at room temperature is no longer than one month and this is a considerable disadvantage from a therapeutic point of view.

Pyridoxal is known to form a Schiff base with many amino acids and its reactivity has led us to examine complexes of pyridoxal with a number of substances in an attempt to find an alternative iron chelator of greater stability than the INH complex and of comparable effectiveness on oral administration. The screening procedures used were the effects on Chang cell iron metabolism (White et al 1976) and on iron excretion in the rat (Hoy et al 1979).

Materials and methods

All chemicals were obtained from Sigma (S), Koch-Light (KL) or British Drug House Ltd (BDH), as indicated in Table 1. Pyridoxal hydrochloride (Sigma) was allowed to react with an equimolar concentration of each substance in 0·1 M HCl in amounts which resulted in 60 mg of the complex being present in 1 ml of solution. There was usually a rapid spectral change in the solution on mixing and in a few cases a precipitate formed. Solutions of pyridoxal-complex formed in this way were later used for oral administration to rats. In

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