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COMMUNICATIONS

Stereospecific inhibition of dopamine-sensitive adenylate cyclase in carp retina by the enantiomers of (±)-1-cyclopropylmethyl-4-(3-trifluoromethylthio-5H-dibenzo [a,d] cyclohepten-5-ylidene) piperidine (CTC)

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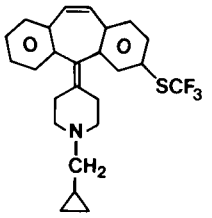
(±)-1-Cyclopropylmethyl-4-(3-trifluoromethylthio-5H-dibenzo [a,d] cyclohepten-5-ylidene) piperidine (CTC,I), a derivative of cyproheptadine, is known to possess both antidopaminergic and anti cholinergic activities (Clineschmidt et al 1979). These activities can be separated, however, when the compound is resolved into its (−)- and (+)-enantiomers. Thus, (−)-CTC is more potent than (+)-CTC in antagonizing apomorphine-induced stereotypy in rodents, in elevating striatal homovanillic acid concentrations and in inhibiting the binding of the dopaminergic antagonists [^{3}H]spiperone and [^{3}H]haloperidol to striatal membranes (Clineschmidt et al 1979; Seeman et al 1979). In contrast, (+)-CTC is more active than (−)-CTC in inhibiting the binding of [^{3}H]quinuclidinyl benzilate (QNB), a potent muscarinic antagonist, to brain homogenates (Clineschmidt et al 1979).



It is now well established that dopaminergic areas of the central nervous system (c.n.s.) possess dopamine-sensitive adenylate cyclase activity and that this system provides a useful model for the study of the interaction of drugs with central dopamine receptors (Iversen 1975). In the present study the effects of the enantiomers of CTC were examined on the recently described dopamine-sensitive adenylate cyclase system present in homogenates of the teleost retina (Watling et al 1980).

Mirror or common carp (*Cyprinus carpio*) of 10–20 cm overall length, were kept at room temperature (20°C) in a tank filled with aerated water. Before an experiment, carp were dark adapted for 10–30 min and decapitated under dim red light to inhibit the migration of screening pigment into the retinal cell layers. Following enucleation and hemisection of the eyes, retinae were separated from pigment epithelium using a small spatula.

Adenylate cyclase activity was assayed in homogenates of carp retinae using the method of Kebabian et al (1972). Cyclic (c) AMP levels were determined by the method of Brown et al (1972), and protein concentrations were measured according to Lowry et al (1951). In agreement with previously reported data (Watling et al 1980), a maximally stimulating concentration of dopamine (100 μM) induced an approximately 6-fold increase in c AMP production (see Fig. 2). The concentration of dopamine producing half-maximal stimulation (EC50 value) was approximately 1 μM.

The inhibition of dopamine-sensitive adenylate cyclase activity by the enantiomers of CTC was initially examined in experiments where the concentration of either (−)- or (+)-CTC was varied whilst the dopamine concentration was held constant at 100 μM. Increasing concentrations of (−)-CTC resulted in a dose-dependent inhibition of the normal maximum dopamine response as shown in Fig. 2. Significant inhibition was apparent at a concentration of 0·1 μM (−)-CTC whilst a concentration of 30 μM (−)-CTC completely abolished the dopamine response. These data yielded an IC50 value for (−)-CTC (concentration of (−)-CTC producing a 50% inhibition of the maximum dopamine response) of 2·2 μM. Application of kinetic analysis to these data, assuming competitive inhibition, indicated a K_I value (inhibition constant) for (−)-CTC of $2{\cdot}2 \times 10^{-8}$ M. In marked contrast, (+)-CTC at concentrations up to 100 μM, only weakly antagonized the normal dopamine stimulation (Fig. 2). Indeed, its inability to induce a 50% inhibition of the dopamine response did not permit the calculation of an inhibition constant.

The inhibitory effect of (−)-CTC on carp retinal dopamine-stimulated adenylate cyclase activity was also examined in an experiment where the normal dopamine dose-response curve was repeated in the presence of a single fixed concentration of (−)-CTC, as shown in Fig. 3. In the absence of (−)-CTC the EC50 for dopamine stimulation of adenylate cyclase activity was 0·78 μM. However, in the presence of a low concentration of (−)-CTC (1 μM), higher concentrations of dopamine were required to activate the retinal adenylate cyclase. This resulted in a parallel shift to the right of the dopamine dose-response curve, although a similar maximum response to that observed in the absence of 1 μM (−)-CTC was apparent. In the presence of 1 μM (−)-CTC the EC50 for dopamine was increased to 18 μM. These data suggest that (−)-CTC acts as a

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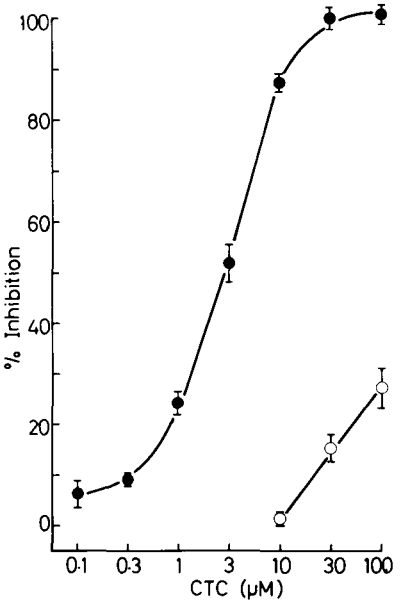

FIG. 1. Inhibition of dopamine stimulated adenylate cyclase activity in carp retinal homogenates by (−)-CTC (●) and (+)-CTC (○). Each point is the mean of two separate experiments involving quadruplicate determinations, and is given with the s.e.m. Results are expressed as per cent inhibition of the normal maximum response produced by 100 μM dopamine. A K_I value for (−)-CTC of $2{\cdot}2\times10^{-8}$ M was calculated from the relationship $K_I = IC50/(1+S/Km)$, where IC50 is the concentration of (−)-CTC required to produce 50% inhibition of the maximum dopamine-induced response, S is the concentration of dopamine used (100 μM) and Km is the concentration of dopamine producing half-maximal stimulation of adenylate cyclase (1 μM). Mean basal cAMP production was $8{\cdot}2\pm0{\cdot}52$ pmol cAMP mg^{-1} protein min^{-1} (n = 8) rising to $49{\cdot}6\pm4{\cdot}7$ pmol cAMP mg^{-1} protein min^{-1} (n = 8) in the presence of 100 μM dopamine.

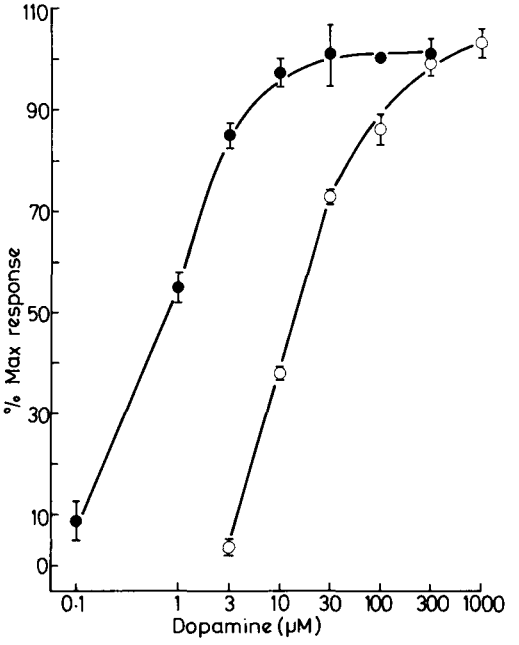

FIG. 2. Effect of dopamine in the presence (○) and absence (●) of 1 μM (−) CTC, on adenylate cyclase activity in a homogenate of carp retina. Each point is the mean of one experiment involving quadruplicate determinations and is given with the s.e.m. Results are expressed as per cent maximum response elicited by 100 μM dopamine. A K_I value for (−)-CTC of $4{\cdot}5\times10^{-8}$ M was calculated from the relationship $K_I = I/(Km'/Km-1)$ where Km′ and Km are the EC50 values for dopamine in the presence and absence of (−) CTC respectively, and I is the concentration of (−) CTC used. Mean basal cAMP production was $7{\cdot}2\pm1{\cdot}9$ pmol cAMP mg^{-1} protein min^{-1} (n = 4) rising to $45{\cdot}6\pm5{\cdot}2$ pmol cAMP mg^{-1} protein min^{-1} (n = 4) in the presence of 100 μM dopamine.

competitive inhibitor in this system and application of the 'dose ratio' indicates a K_I value of $4{\cdot}5\times10^{-8}$ M. This value is in good agreement with that obtained in the previous experiments when the concentration of (−)-CTC was varied whilst that of dopamine was held constant.

The present results suggest that (−)-CTC, with a K_I value of $2{\cdot}2-4{\cdot}5\times10^{-8}$ M, is a potent antagonist of dopamine-sensitive adenylate cyclase, whereas (+)-CTC with a K_I value in excess of 1×10^{-6} M is essentially inactive. When compared with previously obtained K_I values for antagonists on the carp retinal dopamine-sensitive adenylate cyclase (Watling et al 1980), (−)-CTC is approximately equipotent with (+)-butaclamol (K_I value $2{\cdot}2\times10^{-8}$ M). These data provide further evidence for the stereospecific antidopaminergic properties of (−)-CTC and suggest that the enantiomers of CTC may provide a useful pair of compounds for the further study of dopamine receptors in the c.n.s.

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