INTERACTION OF MOLECULES OF THE PHENCYCLIDINE SERIES WITH CARDIAC CELLS

Association with the muscarinic receptor

Michel FOSSET, Jean-François RENAUD, Marie-Cécile LENOIR, Jean-Marc KAMENKA+, Patrick GENESTE+ and Michel LAZDUNSKI

Centre de Biochimie, Faculté des Sciences, Parc Valrose, 06034 Nice Cedex and *+Laboratoire de Chimie Organique Physique Appliquée, Ecole Nationale Supérieure de Chimie, 8 rue Ecole Normale, 34075 Montpellier Cedex, France*

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1. Introduction

Phencyclidine [1-phenyl cyclohexylpiperidine] was introduced in the late 1950s as an intravenous general anesthetic that was non-toxic and non-inflammable. The clinical advantages were unfortunately offset by its prolonged duration of action and psychotomimetic effects [1], properties that have contributed to the emergence of phencyclidine as a major drug of abuse in the United States [2]. Phencyclidine produces long lasting psychosis thought to resemble schizophrenia more than any produced by other psychotomimetics [1]. Phencyclidines have been expected for several years to interfere with the recognition of neurotransmitters at the level of their respective receptors [3,4]. They have been shown to interfere effectively with recognition processes at the level of the muscarinic and of the opiate receptors in the brain [5]. Since it is well known that the pharmacological properties of the acetylcholine receptor in the heart are those of a receptor of the muscarinic type [6], we have decided to analyse the structure–function relationships of a series of phencyclidines in their action on cardiac cells in culture. Cultured cardiac cells have the advantage of being non-innervated. It is only with such a system that one can be sure that the observed effects of drugs are not of pre-synaptic nature. The results presented here show that molecules of the phencyclidine series when tested on the cardiac cell turn out to be agonists of the cholinergic muscarinic receptor with some interesting properties.

2. Materials and methods

2.1. *Ventricle heart cells*

They were cultured from day 11 chick embryos according to [7]. Cells were used 4 days after plating. Under these conditions, cardiac cells form monolayers. They were also cultivated in the form of reaggregates. In this case, dissociated cells were plated into bacterial dishes (Falcon 1008 Petri dish). Spontaneous reaggregation occurs with the first 24–48 h culture. Reaggregates were used 3 days after plating. Beating rates and amplitude of reaggregated cells were recorded as in [8]. Until the reaggregates had reached a stable beating frequency, they were continuously perfused by a standard medium (Hepes–Earle solution) at pH 7.4 and 37°C. Then, phencyclidine, atropine and acetylcholine were perfused to study their action on the beating properties. Studies with acetylcholine were done in presence of 10 μM eserine.

2.2. *Binding of [^{3}H]quinuclidinyl benzilate (QNB) to heart cells in culture*

The properties of binding [^{3}H]QNB, a muscarinic antagonist, were analysed as in [9,10]. Bindings were done directly in the Petri dish for monolayers and on homogenates for reaggregated cells [11]. The competing molecule at the adequate concentration was incubated for 30 min in Earle–Hepes medium at pH 7.4, 37°C, with the cardiac cells prior to addition of 0.9 nM [^{3}H]QNB for monolayers or 0.4 nM [^{3}H]QNB for reaggregates. After another 35 min incubation, monolayers were washed 4 times at room

temperature with 1.5 ml incubation medium. The total washing time was < 15 s. No dissociation occurs during washing near 22°C. Washed cells were then suspended in 0.1 N NaOH and the bound radioactivity was measured using Picofluor. [^{3}H]QNB binding on homogenates of reaggregated cardiac cells was according to [11]. The specific binding of [^{3}H]QNB to cardiac cells in culture is the amount of binding which is prevented by concentration of non-radioactive atropine which saturates the muscarinic receptor (10 μM) or by saturating concentrations of the phencyclidine derivatives. Under our experimental conditions, the amount of specific binding compared to total binding is 65–70% for monolayers and 95% for homogenates of reaggregates. All binding curves were fitted according to [12].

2.3. *Chemicals*

Molecules of the phencyclidine series were synthesized according to the methods in [13]. Other chemicals were from standard sources.

3. Results

3.1. *Negative inotropic and chronotropic effect of phencyclidine (GK1) compared to the effect of acetylcholine on cardiac reaggregated cells*

Figure 1A shows that acetylcholine triggers a negative inotropic and negative chronotropic effect and stops the beating of cardiac cell reaggregates. Figure B shows the effect of phencyclidine (GK1) on the beating properties of the reaggregated cells. Phencyclidine gives the same negative inotropic and chronotropic effect as acetylcholine. GK1 acts in a range of concentrations similar to the range of concentrations necessary to see acetylcholine action. However, in opposition with acetylcholine (fig.1A), reaggregates treated by phencyclidine (fig.1B) do not desensitize. Figure 1C shows that the negative chronotropic and inotropic effect of GK1 is completely prevented when the reaggregates are preincubated with atropine. A dose–response curve for GK1 is shown in fig.2A and compared to the dose–response curve given with acetylcholine. GK1 acts at 0.2–30 μM with a half-maximum effect at 0.4 μM. The dose-response curve for the protection of the effects of GK1 by atropine is given in fig.2B. Atropine by itself

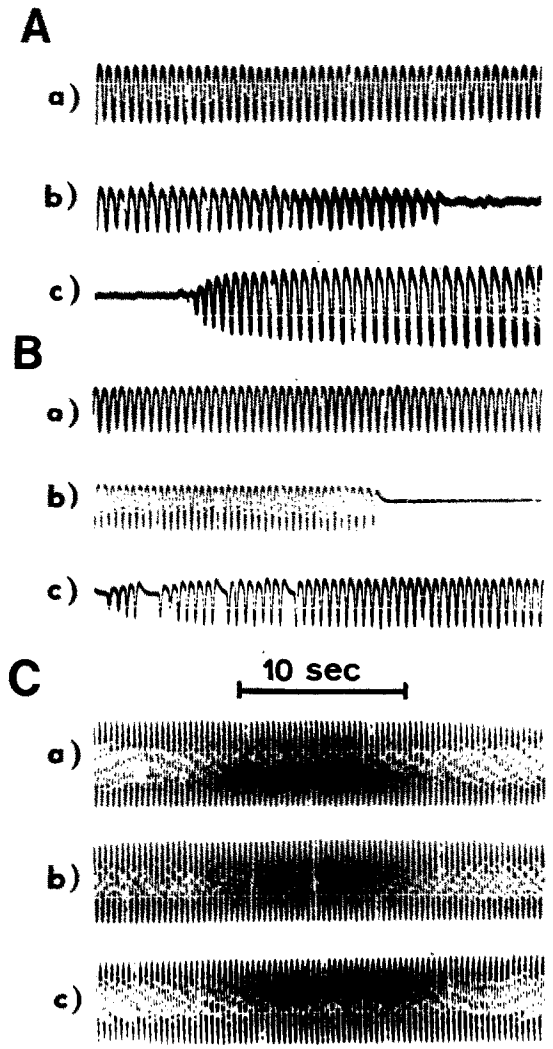


Fig.1A. Effect of acetylcholine on the rate and amplitude of beating of reaggregated cardiac cells of embryonic chick ventricles. (a) Control beating rate recorded from one reaggregate. (b) Addition of 10 μM acetylcholine decreases the rate and amplitude of beating; then after 1.20 min the beating is stopped. (c) Desensitization occurs 3 min later.
Fig.1B. Effect of GK1 on the rate and amplitude of beating of reaggregate cell cultures. (a) Control beating rate recorded from one reaggregate. (b) Addition of 30 μM GK1 decreases the rate and amplitude of beating; after 5.5 min, beatings are stopped as long as reaggregates are in the presence of GK1 (10 min). (c) On washing recovery occurs after 5 min.
Fig.1C. Protection of the effect of GK1 on reaggregated cell cultures. (a) Control beating rate recorded from one reaggregate bathed in Earle–Hepes solution containing 0.01 μM atropine. (b) 5 min after addition of 30 μM GK1 + 1 μM atropine. (c) 10 min later.

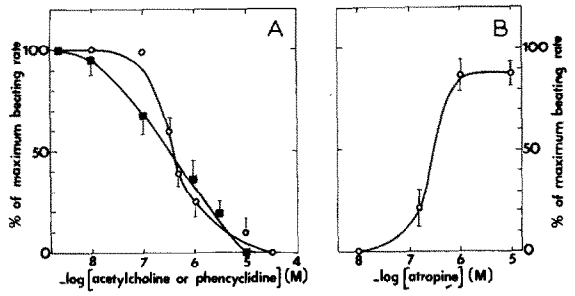


Fig.2A. Beating-response curves for GK1 (○) and acetylcholine (■), on reaggregated heart cells. Acetylcholine and phencyclidine trigger a negative inotropic and chronotropic effect and stop beating at 10 μM and 30 μM, respectively. The minimum beating rate at a given dose was plotted as percentage of the control rate. Fig.2B. Dose–response curve of protection of the effects of GK1 on reaggregated heart cells by different concentrations of atropine. GK1 is used at a blocking concentration (30 μM), atropine at 0.01–10 μM. Maximum effect of atropine is observed at 1 μM. The maximum beating rate at a given dose was plotted as percentage of the control rate before addition of GK1. The bars in fig.2 correspond to ± SEM (n = 5–7).

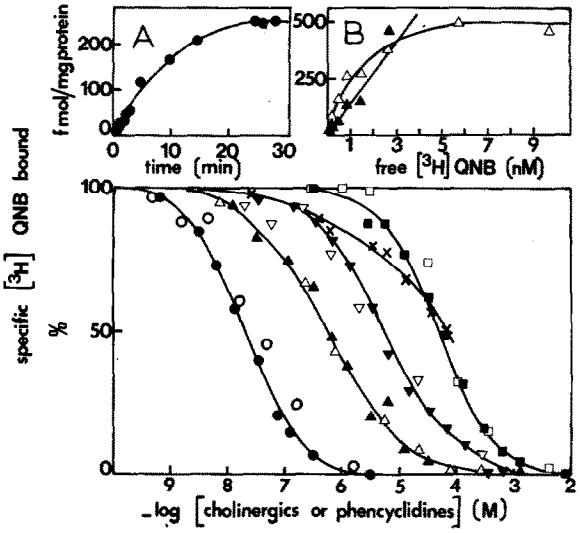


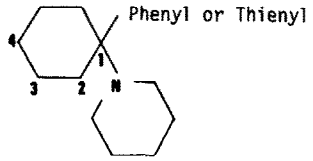
Fig.3. Inhibition by molecules of the phencyclidine series and by cholinergic muscarinic antagonists and agonists of [^{3}H]QNB binding to monolayers and reaggregated cultures of cardiac cells. Binding was carried out at 37°C with 0.9 nM [^{3}H]QNB (monolayers, black symbols) or 0.4 nM (reaggregates, open symbols). (●, ○) atropine, (▲, △) GK4, (▼, ▽) oxotremorine, (■, □) GK1 and (X) acetylcholine. Cells were incubated 20 min with 10 μM eserine before addition of acetylcholine. Inset A: Time course of specific association of 0.9 nM [^{3}H]QNB to cardiac cells in monolayer culture (pH 7.4, 37°C). Inset B: Binding of [^{3}H]QNB to cardiac cells in monolayer culture. Increasing amounts of [^{3}H]QNB (44 Ci/mmol) were incubated at 37°C. (△) specific binding, (▲) non-specific binding in the presence of 10 μM atropine. Ordinates: [^{3}H]QNB in fmol/mg protein. Maximum specific binding is 500 fmol/mg protein and apparent equilibrium dissociation constant is $K_{0.5}$ = 0.9 nM for monolayers. Maximum specific binding is 170 fmol/mg protein and $K_{0.5}$ = 0.4 nM for reaggregates (results not shown). Each point in this figure represents the average of duplicate experiments.

is without any effect on beating frequency. An important property differentiates GK1 from acetylcholine: with acetylcholine, there is a very rapid desensitization of the chronotropic and inotropic effect even in presence of eserine. The desensitization process does not exist with GK1. When the reaggregates are stopped, they do not recover their beating properties even after periods as long as 30 min GK1 perfusion. However the reversibility of the effect of GK1 is observed after 5–10 min washing.

3.2. *Interaction of phencyclidines with the muscarinic receptor of embryonic cardiac cells in reaggregate and monolayer cultures*

As shown in fig.3, non-radioactive acetylcholine, oxotremorine and atropine in increasing concentrations compete with the muscarinic antagonist [^{3}H]QNB for the association to the muscarinic receptor. Molecules of the phencyclidine series also compete with [^{3}H]QNB for the muscarinic receptor. IC_{50} values for a series of molecules of the phencyclidine series are presented in table 1. IC_{50} values range from 0.7–200 μM. It is interesting to remark that similar results are observed with cells in monolayers and in reaggregates (fig.3). It is well known that chick embryo cells cultivated in monolayers are insensitive to acetylcholine in their beating properties [14]. Clearly these cells in monolayers have a muscarinic receptor with all the properties of a functional receptor for binding of agonists and antagonists. However this receptor is most probably not coupled to the cation ionophores which regulate the chronotropic and inotropic properties of the normal cardiac cell. It is interesting to remark that if phencyclidine (or its analogues) is indeed a muscarinic agonist, one should observe binding of these molecules to the muscarinic receptor in cardiac cell monolayers but, similarly to

Table 1
Inhibition by phencyclidines of [^{3}H]QNB bound, at equilibrium, to the muscarinic receptor of heart cells in monolayer culture



Code	Nomenclature of phencyclidine analogs	[a]IC_{50} (μM)	[b]K_d (μM)	n_H
GK0	1-(2-thienyl)cyclohexylpiperidine	31	16	1.0
GK1	1-phenyl cyclohexylpiperidine (phencyclidine)	55	28	0.9
GK2	1-phenyl *c*-4-tert-butyl *r*-cyclohexylpiperidine	> 200		
GK3	1-phenyl *t*-4-tert-butyl *r*-cyclohexylpiperidine	6.9	3.5	1.0
GK4	1-phenyl *c*-4-methyl *r*-cyclohexylpiperidine	0.7	0.4	0.8
GK5	1-phenyl *t*-4-methyl *r*-cyclohexylpiperidine	16	8	0.9
GK6	1-phenyl *c*-3-methyl *r*-cyclohexylpiperidine	25	12.5	0.9
GK7	1-phenyl *t*-3-methyl *r*-cyclohexylpiperidine	15	7	0.8
GK8	1-phenyl *c*-2-methyl *r*-cyclohexylpiperidine	19	9	0.8
GK9	1-phenyl *t*-2-methyl *r*-cyclohexylpiperidine	14	7	0.9
GK11	1-(2-thienyl)*c*-2-methyl *r*-cyclohexylpiperidine	40	20	0.9
GK12	1-(2-thienyl)*t*-2-methyl *r*-cyclohexylpiperidine	78	39	0.8
GK14	1-phenyl *t*-2-methoxy *r*-cyclohexylpiperidine	58	29	0.9
GK15	1-phenyl *c*-2-methoxy *r*-cyclohexylpiperidine	8.1	4	0.8
	atropine	0.017	0.009	0.9

[a] IC_{50} is the concentration of unlabeled drug which induces half-displacement of specifically bound [^{3}H]QNB, in the experimental conditions

[b] K_d is the apparent equilibrium dissociation constant of the complex formed between cardiac cells in culture and the unlabeled drug. When the Hill coefficient (n_H) is close to 1.0, K_d is calculated from the IC_{50} value by the formula:

$$IC_{50} = K_d(1 + [^3H]QNB/K_{0.5(QNB)})$$

where [^{3}H]QNB is the concentration of free [^{3}H]QNB at half-inhibition and $K_{0.5(QNB)}$ = 0.9 nM calculated from direct saturation binding curve (see fig.3B)

acetylcholine, no effect on the beating properties. Experimental evidence shows this expectation to be correct.

A number of control experiments were carried out in order to be sure that:

(i) No binding has occurred on the small fibroblast population which always exists in cultures of cardiac cells in monolayers;

(ii) The measured [^{3}H]QNB association to the cell corresponds to binding and not to uptake.

Cultures of chick embryo fibroblast cells do not show any specific binding of [^{3}H]QNB. On the other hand, monolayer cells treated for 1 h with 0.3 mM, 2,4-dinitrophenol to deplete the intracellular store of ATP from 8 mM down to 80 μM [15] display [^{3}H]QNB binding properties identical to those of untreated cells. Since the metabolic inhibitor that blocks uptake systems is without effect on the binding of [^{3}H]QNB and on the competition between phencyclidines and [^{3}H]QNB, it seems reasonable to assume that uptake of QNB is not occurring and that binding is the major feature.

4. Discussion

Phencyclidine provokes both a negative inotropic

and a negative chronotropic effect on chick embryo cardiac cells in culture. These effects are very similar to those induced by acetylcholine. Therefore the experimental observations suggest that phencyclidine is an agonist-like molecule of acetylcholine for the muscarinic receptor of the cardiac cell. This conclusion is confirmed by the fact that atropine at very low doses protects against the effect of the phencyclidine.

The general properties of the interaction of phencyclidine with cardiac cell reaggregates are the following:

(i) Negative chronotropic and inotropic effect with arrest of the beating;
(ii) In contrast to the situation observed with acetylcholine, no desensitization is observed for phencyclidine at low or at high concentrations;
(iii) The action of phencyclidine is reversible after washing;
(iv) The phencyclidine molecule manifests its effect in a range of concentrations similar to acetylcholine;
(v) The beating properties of cardiac cells in monolayers do not respond to phencyclidine, an expected result if phencyclidine acts as agonist of acetylcholine on the muscarinic receptor.

Similarly to oxotremorine, acetylcholine and atropine, phencyclidines antagonize the specific [^{3}H]QNB binding to the muscarinic receptor. The main properties of the association of phencyclidines with the acetylcholine muscarinic receptor are the following:

(i) The dissociation constants (K_d) of the complexes formed between the 14 phencyclidines assayed and the muscarinic receptor range from 0.4 μM to > 39 μM.

The binding of phencyclidines to the muscarinic receptor is characterized by a Hill coefficient near 1 (0.8–1.0), the interaction is therefore of the Michaelian type.

(ii) There are differences between isomeric pairs.

There is a considerable difference in affinity between GK2 and GK3 which are *cis* and *trans* isomers in position 1 of the cyclohexyl ring; there are also differences between GK4 and GK5. GK4 has more affinity for the muscarinic receptor. However the substituted compounds at position 2 or 3 of the cyclohexyl ring of phencyclidines do not differ greatly in their affinity for the muscarinic receptor.

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