Biochemical Pharmacology, Vol. 34, No. 7, pp. 949–954, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

EFFECTS OF PHENCYCLIDINE AND ANALOG DRUGS ON ACETYLCHOLINE RECEPTOR OF CULTURED MUSCLE CELLS

ADINA AMIR*†, PINHAS FUCHS*, AVIVA GAMLIEL‡, MOSHE REIS‡ and ASHER SHAINBERG‡

*Department of Virology, Israel Institute for Biological Research, Ness-Ziona, and ‡Department of Life Sciences, Bar-Ilan University, Ramat-Gan 52 100, Israel

(*Received* 1 *April* 1982; *accepted* 22 *June* 1984)

Abstract—Myotubes grown in culture provided a convenient experimental system for the study of the effects of phencyclidine (PCP) and analog drugs on both acetylcholine receptor (AChR) function and on its binding properties. The extent of PCP retention by these cells was studied on the same preparations. PCP, *N*-ethyl-l-phenylcyclohexylamine (PCE), PCP methiodide (PCPMeI), 1-[1-(3-aminophenyl)-cyclohexyl] piperidine (NH_2PCP) and 1-[1-(2-thienyl)cyclohexyl] piperidine (TCP) were found to inhibit carbamylcholine (CbCh)-induced ^{22}Na and ^{45}Ca ion fluxes with 50% inhibition (I_{50}) at 2–6 μM drug concentration. The I_{50} for CbCh-induced $^{42}K^+$ efflux was 8–20 μM. Ketamine was less efficient with an I_{50} of 100 μM. Binding of [^{125}I] α-bungarotoxin ([^{125}I]α-BGT) was not affected at drug concentrations that cause 100% inhibition of ion fluxes. Retention of [^{3}H]PCP by the myotubes was a saturable process with half-maximal saturation at ~20 μM PCP. It was inhibited by PCP and several tertiary analogs, with an I_{50} of ~ 20 μM. PCPMeI was much less effective, with an I_{50} of 1 mM. PCPMeI was, however, as potent as PCP in its inhibition of the AChR function although the amount retained by the cells was 50-fold lower than that of PCP. These results are consistent with the theory that PCP and analog drugs affect AChR at a site other than the α-BGT binding site, possibly at the ionic channel of the nicotinic receptor.

Phencyclidine (PCP) and similar compounds are potent psychotomimetics and are most popular drugs of abuse [1, 2]. It has been shown that PCP interacts with various receptor systems. A specific PCP receptor was demonstrated in rat brain membranes [3–8]. Maayani and Weinstein [9], however, suggested that this receptor may be an artifact of the binding assay. We have studied the interaction of PCP with the nicotinic acetylcholine receptor (AChR) of cultured myotubes and found that the binding of [^{125}I]α-bungarotoxin ([^{125}I]α-BGT) was not affected by PCP at concentrations which inhibited AChR function [10]. We have, therefore, suggested that PCP affects the AChR at a site other than the α-BGT binding site, possibly the ionic channel of the AChR [11]. This was also shown by several studies which demonstrated a direct interaction of PCP with the ionic channel of the AChR [12–17]. Some of these studies were done on systems in which nicotinic receptor function and effects on α-BGT binding were measured on two different preparations, namely, animal organs for physiological studies of receptor function and membrane preparations for toxin and PCP binding.

In the present work we have attempted to elucidate and localize the effect of PCP and of related drugs on the function of the nicotinic AChR of intact skeletal muscle cells grown in culture. Rat myotubes differentiated *in vitro* provide a convenient system for the simultaneous study of drug effects on receptor function and on receptor binding properties. Moreover, the extent of PCP retention by these cells can be studied on identical preparations of muscle cells.

† Address all correspondence to: Dr. A. Amir, Department of Virology, Israel Institute for Biological Research, Box 19, Ness-Ziona, Israel.

MATERIALS AND METHODS

Chemicals. The following compounds, provided by Dr. A. Kalir, were used: PCP [18, 19]; 1-[1-(2-thienyl)cyclohexyl] piperidine (TCP) [18, 19]; PCP methiodide (PCPMeI) [18]; 1-[1-(3-aminophenyl)-cyclohexyl] piperidine (NH_2PCP) [20]; *N*-ethyl-l-phenylcyclohexylamine (PCE) [20]; and 1-phenylcyclohexylamine (PCA). Ketamine hydrochloride was obtained as a 10 mg/ml solution (Ketalar, Parke Davis & Co., Pontypool, Gwent, U.K.). Other chemicals were purchased from the Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Radioisotopes. $^{45}Ca^{2+}$ (sp. act. 17 mCi/mg Ca^{2+}) was obtained from the New England Nuclear Corp. (Boston, MA, U.S.A.). $^{22}Na^+$ (sp. act. 100 mCi/mg Na^+) was obtained from the Radiochemical Centre (Amersham, U.K.). $^{42}K^+$ (sp. act. 0.240 to 0.300 mCi/mg K^+) was obtained from the Israel Atomic Energy Commission (Soreq Nuclear Research Center). Specifically labeled 3,4-piperidyl[^{3}H]PCP ([^{3}H]PCP) [21] (sp. act. 23.7 Ci/mmole; radiochemical purity 99%) was supplied by the Nuclear Research Center, Negev, Israel. [^{125}I]α-BGT (sp. act. 50–150 Ci/mmole) was prepared by the chloramine T iodination method [22].

Cell cultures. Experiments were carried out on rat myotubes differentiated *in vitro*, 7–10 days after

plating [23]. The cells were cultured in collagen-coated 30 mm plastic tissue culture plates (Nunc) or in Linbro Multiwell Tissue Culture trays (24 × 16 mm wells, 2 cm^2 each). A batch of 7- to 10-day-old myotube cultures were first examined microscopically to ensure morphological differentiation and uniformity of sister cultures. Cultures of the same batch were used for binding and for ion flux measurements. Seven- to ten-day-old cultures in 30 mm diameter dishes contained 1.3 ± 0.1 mg protein and about 200 fmoles α-BGT binding sites.

[^{125}I]αBGT binding. The level of the receptor was measured by exposing intact muscle cultures to 6×10^{-8} M [^{125}I]α-BGT (50–150 Ci/mmole) for 1 hr in the growth medium. Non-bound toxin was removed by six washes with 2 ml of phosphate-buffered saline (PBS) containing 1 mg/ml bovine serum albumin (BSA). Bound toxin was measured by laying dishes on a flat crystal 2-inch diameter γ detector (Elscint, Haifa, Israel). Counting efficiency was 28%.

PCP retention. We shall use the term retention for the binding of [^{3}H]PCP to the cells, since the retention may represent binding and/or uptake into cellular compartments. The retention of [^{3}H]PCP by muscle cells was studied on 8- to 10-day-old monolayer cell cultures in the dish. The growth medium was removed by aspiration and the cells were covered with sucrose–histidine buffer (SH-buffer) (at 22° or 37°) containing [^{3}H]PCP or other drugs, as specified. The SH-buffer used contained; 300 mM sucrose, 40 mM histidine and 2 mM $CaCl_2$, pH 7.4. The cells were then incubated at 22° or 37° for various periods of time. The process was stopped by removal of the incubation medium, followed by three washes with SH-buffer at 0°. The total wash sequence was completed in 8–10 sec/culture. The cells were in contact with the washing buffer for 5–6 sec only. Increasing the number of washes from three to six had no effect on [^{3}H]PCP retention. Cells were then lysed by 1% sodium dodecyl sulfate (SDS) and the lysate was used for both protein determination and radioactivity measurements. Protein was determined by the method of Lowry *et al.* [24]. Radioactivity was determined in a Packard Tricarb scintillation counter in 10 ml scintillation fluid [1 liter toluene, 0.5 liter Triton X-100, 0.45 g dimethyl-POPOP (1,4-bis-[2-(4 methyl-5-phenyloxazolyl)] benzene), and 8 g PPO (2,5-diphenyloxazole)] containing 1 ml of sample (about 40% counting efficiency).

Ion flux measurements. The effects of drugs on carbamylcholine (CbCh)-induced influx of $^{45}Ca^{2+}$ or $^{22}Na^+$ were measured as follows. Drugs were added to the growth medium and cells were incubated with the drugs at 37° for 10 min. The growth medium was then replaced for 1 min by 1 ml of the ion-free SH-buffer containing the drug, in order to remove the adhering ions. The medium was then replaced by 1 ml of SH-buffer containing 0.5 μCi radioisotope and the drug. Influx was induced by the addition of (CbCh) to a final concentration of 1 mM. After swirling for 30 sec at 22–25°, the radioactive solution was removed by aspiration, and cells were washed and collected for radioactivity counting.

The effects of drugs on CbCh-induced $^{42}K^+$ efflux were measured as follows. Cells were loaded with $^{42}K^+$ by adding 50 μCi/ml of the isotope into the growth medium and incubating the cultures, at 37°, for 2.5 to 3 hr. $^{42}K^+$ concentration inside the cells was measured every 30–60 min during loading. Saturation occurred after 2.5 hr. At the end of the loading period, the drugs were added to each culture, and incubation was continued for 10 min. The cultures were then transferred to room temperature, the growth medium was removed, and the cultures were rinsed once with SH-buffer and incubated in 1 ml of this buffer containing the respective drug with or without CbCh. Samples (0.1 ml) from the buffer were collected for counting after 10 sec, 20 sec, 1 min, 10 min and 20 min. Controls underwent similar treatment using SH-buffer without the drug. No agonist effects of the drugs were found.

Each experiment was repeated at least twice, and experimental points represent an average of two to three replicates. The reproducibility of the replicates was usually 5–10%.

RESULTS

Inhibition of ion fluxes. The effects of PCP on CbCh-induced ion fluxes and on α-BGT binding are shown in Fig. 1. A dose-dependent inhibition of both $^{22}Na^+$ and $^{45}Ca^{2+}$ uptake was caused by PCP. The concentration of PCP giving a 50% inhibition (I_{50}) was 2–6 μM. In some experiments, PCP exerted a biphasic effect on $^{45}Ca^{2+}$ uptake. At 0.1 μM PCP a 24% potentiation of CbCh-induced $^{45}Ca^{2+}$ uptake was observed (Fig. 2). [^{125}I]α-BGT binding was not affected by PCP at concentrations up to 100 μM, but was 30 and 42% inhibited by 1 mM PCP and PCPMeI respectively. This suggests that PCP, at concentrations up to 100 μM, does not react with the binding sites for α-BGT.

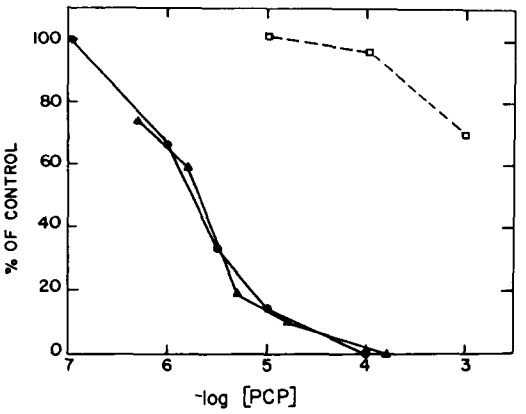

Fig. 1. Effect of PCP on acetylcholine receptor function and on α-BGT binding. Acetylcholine receptor function in myotubes grown in 30 mm plates was measured by $^{22}Na^+$ or $^{45}Ca^{2+}$ uptake in response to 1 mM carbamylcholine. One hundred percent CbCh-induced $^{45}Ca^{2+}$ uptake = 130 nmoles/plate/min. The basal uptake in the CbCh was 8 nmoles/plate/min. For $^{22}Na^+$ were 163 and 6 nmoles/plate/m dred percent bound α-BGT w (□---□) α-BGT binding; (▲— (●—●) $^{45}Ca^{2+}$ u

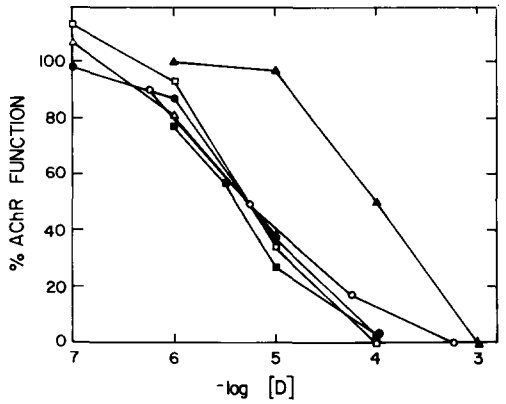

Fig. 2. Inhibition by PCP and by various analogs of acetylcholine receptor function. Log dose effect tests of PCP, NH_2PCP, PCPMeI, TCP, PCE and ketamine on CbCh-induced $^{45}Ca^{2+}$ uptake in myotubes. Values for uninhibited ion fluxes were similar to those of Fig. 1. Key: (□—□) PCP; (■—■) NH_2PCP; (○—○) PCPMeI; (●—●) TCP; (▲—▲) ketamine; and (△—△) PCE.

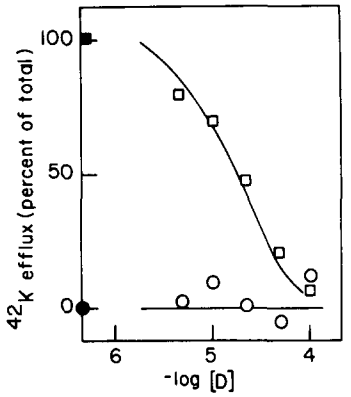

Fig. 4. Inhibition of PCP and PCP analogs of carbamycholine-induced $^{42}K^-$ efflux. Myotubes were preloaded with $^{42}K^+$ for 2.5 to 3 hr. Following wash with SH-buffer, the cells were treated with the drugs in the presence or absence of CbCh. Samples (0.1 ml) were taken for counting at different times. Results depicted here are for a 20-min incubation with 1 mM CbCh. The results obtained for PCP, TCP and PCE were identical. Key: basal efflux: (●) [CbCh] = 0, [D] = 0; (■) CbCh-induced efflux: [CbCh] = 1 mM, [D] = 0; (○—○) drug effect on basal efflux: [CbCh] = 0, [D] = PCP, TCP or PCE; and (□—□) drug effect on CbCh-induced efflux; [CbCh] = 1 mM, [D] = PCP, TCP or PCE.

The inhibition by PCP and by various PCP analogs of CbCh-induced $^{45}Ca^{2+}$ uptake is shown in Fig. 2. PCP and all the PCP analogs tested show a dose-dependent inhibition of the ion fluxes with I_{50} of 2–6 μM. Ketamine, a drug pharmacologically related to PCP, was a less efficient inhibitor with an I_{50} of 100 μM.

The effect of PCP on the dose–response curve for CbCh-induced $^{45}Ca^{2+}$ uptake was studied for two concentrations of PCP; 2 μM and 5 μM. The results of this experiment are shown in Fig. 3. PCP caused a shift of the dose–response curve. At high CbCh concentrations, the AChR function in the presence of PCP did not reach the maximal values as obtained without PCP.

CbCh induces an increased initial rate of $^{42}K^+$ efflux. Due to the desensitization of the AChR in the presence of CbCh [25], the rate of $^{42}K^+$ release decays within seconds and equals the rate of release from untreated control cells. Therefore, the effects of PCP and analog drugs on CbCh-induced $^{42}K^+$ efflux measured at 20 min after addition of CbCh was similar to that at 30 or 60 sec after its addition. The results of an experiment measuring the effect of PCP on CbCh-induced $^{42}K^+$ efflux are depicted in

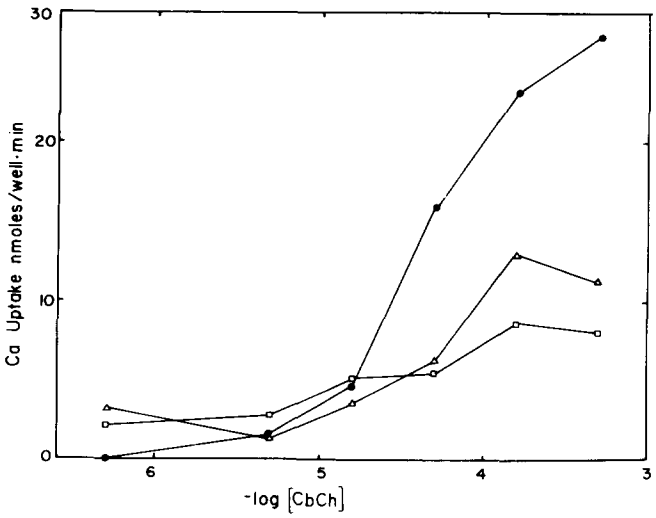

Fig. 3. Dose–response curve for carbamylcholine induced $^{45}Ca^{2+}$ uptake in the presence of PCP. AChR function was measured by $^{45}Ca^{2+}$ uptake in response to various concentrations of CbCh in the presence of PCP. Key: (●—●) 0 PCP; (△—△) 2×10^{-6} M PCP; and (□—□) 5×10^{-6} M PCP.

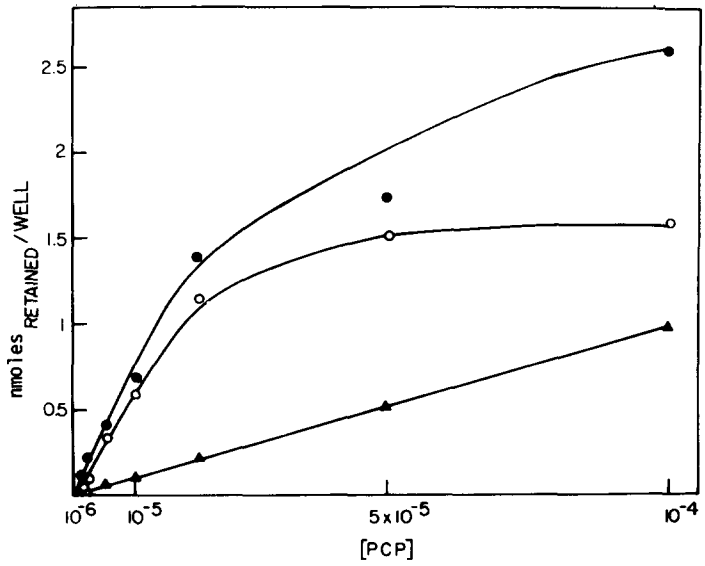

Fig. 5. Retention of PCP by myotubes. Mytotubes grown in 16 mm wells (0.37 mg protein/well) were treated for 10 min at 22° with [^{3}H]PCP (sp. act. 0.56 Ci/mmole). Non-specific retention was measured in the presence of 10^{-3} M PCP. Key: (●—●) total retention; (▲—▲) non-specific retention; and (○—○) specific retention.

Fig. 4. PCP, TCP and PCE were equally potent in inhibiting the CbCh-induced $^{42}K^+$ efflux. The I_{50} for this inhibition was 7–20 μM. PCP, TCP and PCE had no effect on the basal $^{42}K^+$ efflux (when no CbCh was added) at concentrations up to 10^{-4} M and for incubation periods of as long as 60 min. PCA induced a leak of $^{42}K^+$ of about 30% in 20 min. Therefore, the inhibitory effects of PCA on CbCh-induced $^{42}K^+$ efflux were difficult to measure.

Retention of [^{3}H]PCP by myotubes. Retention of [^{3}H]PCP by cultured myotubes was linear with the number of cells in the dish. (The number of cells in such cultures is determined by the size of the culture plate.) Figure 5 shows the total retention of [^{3}H]PCP measured at constant specific activity, using [^{3}H]PCP of 0.56 Ci/mmole. The curve was resolved in two components: a linear nonsaturable retention, determined in the presence of 10^{-3} M unlabeled PCP (non-specific retention) and a saturable component, calculated by subtracting the linear from the total retention. The capacity of this saturable component was ~ 4 nmoles/mg protein, at 22°, and the PCP concentration for half-maximal saturation was about 2×10^{-5} M. The inhibition of retention of [^{3}H]PCP by unlabeled PCP was also determined. Figure 6 shows the inhibition by PCP of the retention of 8×10^{-8} M [^{3}H]PCP. The I_{50} value was about 2×10^{-5} M at 22°, in good agreement with results from direct binding experiments. TCP and NH_2PCP were as efficient as PCP in inhibiting the [^{3}H]PCP retention by the cells. PCPMeI, however, was much less efficient with an I_{50} of 10^{-3} M [11]. Figure 7 shows the association kinetics of [^{3}H]PCP to the myotubes. In a binding system where the law of mass action is valid, the kinetic constants can be calculated from the following equation:

$$\ln[B_{eq}/(B_{eq} - B_t)] = k_{ob}t \qquad (1)$$

where B_{eq} is the specific retention at equilibrium and B_t is the specific retention at time t, k_{+1} is the rate of association, k_{-1} is the rate of dissociation and $k_{ob} = k_{+1} \cdot [D]_{total} + k_{-1}$. The insert in Fig. 7 is drawn according to equation 1. k_{+1} and k_{-1} were calculated from the slope and the intercept of k_{ob} as a function of PCP concentration. k_{+1} and k_{-1} thus calculated were $0.28 \pm 0.05 \times 10^4\ M^{-1}\ min^{-1}$ and $0.12 \pm 0.02\ min^{-1}$ respectively. The ratio of k_{-1}/k_{+1} was $\sim 4 \times 10^{-5}$ M, in good agreement with I_{50} values from equilibrium experiments.

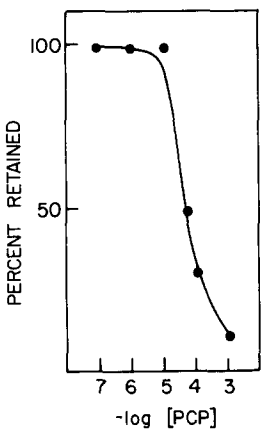

Fig. 6. Inhibition of [^{3}H]PCP retention by PCP. Myotubes, grown in 16 mm diameter wells, were incubated for 20 min with 0.5 ml of 8×10^{-8} M [^{3}H]PCP (600,000 cpm) and various concentrations of PCP. One hundred percent retention was ~ 150,000 cpm/well.

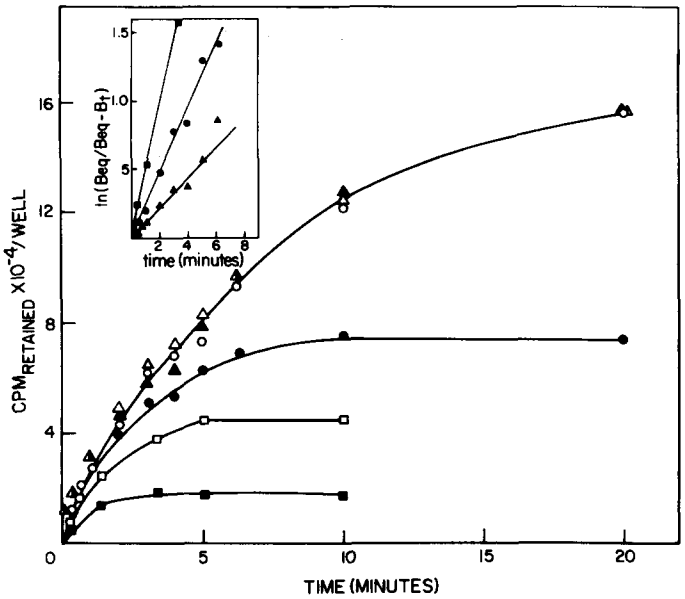

Fig. 7. Kinetics of [^{3}H]PCP retention by myotubes. Myotubes grown in 16 mm diameter wells (~ 0.37 mg protein/well) were incubated with 0.5 ml of 8×10^{-8} M [^{3}H]PCP (600,000 cpm) and various concentrations of PCP. Insert: Calculated from data presented in the figure. B_{eq} is specific retention at equilibrium, B_t is specific retention at time t. Key: (▲—▲) 8×10^{-8} M PCP; (△—△) 10^{-6} M PCP; (○—○) 10^{-5} M PCP; (●—●) 5×10^{-5} M PCP; (□—□) 10^{-4} M PCP; and (■—■) 10^{-3} M PCP.

DISCUSSION

Several recent studies have suggested that PCP and related drugs interact with the ionic channel of nicotinic receptors [10–17]. Tsai *et al.* [12] studied effects of PCP on electrical excitability and chemosensitive properties of amphibian and mammalian skeletal muscles. These studies were complemented by biochemical studies on preparations of fish electric organs [12–14], where it was shown that, although PCP does not inhibit the interaction of acetylcholine or of α-BGT with the nicotinic receptors, it alters the properties of the ionic channel of the AChR and inhibits the binding of perhydrohistrionicotoxin. Direct binding studies of [^{3}H]PCP to a membrane preparation of torpedo electric organs performed by Kloog *et al.* [14] showed that [^{3}H]PCP binds to a single class of binding sites with an equilibrium dissociation constant of 7.5 ± 2.5 μM, possibly on the cholinergic ionophore.

Cultured myotubes can serve as a model for postsynaptic events occurring at the neuromuscular junction [25]. Ion fluxes were induced in these cultures by CbCh. These ion fluxes were inhibited by α-BGT with an I_{50} of 5×10^{-9} M, indicating their nicotinic character. Tetrodotoxin, at concentrations far higher than those required to saturate the action potential sodium-channels, has no effect on agonist-induced $^{45}Ca^{2+}$ uptake (see p. 73 of Ref. 26). Moreover, in the ion-free SH-buffer no action potential was obtained after CbCh application. These findings indicate that $^{45}Ca^{2+}$ influx, induced by CbCh, is mediated by the receptor ion-channel and not by the voltage-dependent sodium-channels (manuscript in preparation).

We have therefore used this experimental system to study the binding characteristics of PCP and analog drugs to myotubes grown in culture and the effects of these drugs on the nicotinic receptor function, i.e. on ion fluxes through activated receptor ion-channels.

Our results show a 100% inhibition of CbCh-induced ion fluxes by drug concentrations that do not affect α-BGT binding. The effect of PCP on the dose–response curve for CbCh-induced $^{45}Ca^{2+}$ uptake (Fig. 3) is consistent with a non-competitive inhibition. This indicates that PCP and analog drugs interact with the AChR at a site(s) different from the α-BGT binding site, possibly the cholinergic ionophore.

All the PCP analogs tested, including PCPMeI, were equally potent in the inhibition of ion fluxes. This is in agreement with the results of Albuquerque *et al.* [17] and may show that the effects of PCP and analog drugs on the receptor ion-channel are not related to behavioral potencies of these drugs. In this respect, the interaction with the receptor ion-channel differs from the interaction of the PCP-like drugs with the action potential channels. In the latter case, a correlation between K^+ conductance and behavioral potency was demonstrated [17].

PCPMeI is an interesting compound, since it is a quaternary analog of PCP which does not penetrate biological membranes [13]. Recently it has been used as such to differentiate between internal and external sites of action at the nicotinic acetylcholine receptor channel complex [27]. Our results indicate that with AChR ion channels the site of action of PCP and analog drugs is externally located (Fig. 2).

To further characterize the interaction of PCP with

cultured myotubes, we performed direct binding studies using [^{3}H]PCP. The binding experiments were performed on intact cells in their dishes. We found that myotubes retained [^{3}H]PCP by a saturable process. At a concentration of 4 μM PCP (which is the I_{50} for inhibition of AChR function by this compound), the number of PCP molecules retained was 3–4 orders of magnitude larger than the number of α-BGT binding sites. In cellular homogenates, a ratio of 1 : 3 between [^{3}H]PCP and [^{125}I]α-BGT binding was found [14]. This high retention of [^{3}H]-PCP relative to existing α-BGT binding sites obscured any specific binding to the AChR. Thus, the direct binding experiments performed on intact myotubes are not directly related to the AChR, but characterize the retention, affinity and capacity of sites present on intact cells. The poor ability of PCPMeI to displace [^{3}H]PCP bound to the myotubes [11] may be explained by its limited penetration through the cell membrane. The high capacity and low affinity for PCP retention seem to be general properties of intact cells [28]. In intact neuroblastoma cells we have shown that retention was temperature-dependent and was inhibited by 2,4-dinitrophenol (DNP), which may indicate that in these cells retention represents (at least in part) an active uptake of PCP into the cells [28]. However, this seems not to be the case in our system of myotubes grown in culture, since PCP retention was practically the same at 22° and at 4°, or in the presence of 10^{-3} DNP and 10^{-5} M iodoacetate (data not shown).

PCP inhibition of the AChR-controlled ion flux could be explained by different mechanisms. One possible mechanism would be binding to high affinity sites distinct from α-BGT binding sites such as found for various torpedo species [29]. Mittag and Gross [16] proposed an indirect desensitization of the AChR due to inhibition of AChE by PCP. Since we used CbCh (which is AChE-insensitive), this possibility is excluded in our case. These authors and others [30] also propose that PCP increases the rate of receptor inactivation (desensitization). This mechanism is also consistent with our results. Another possibility would be that PCP interacts with intracellular or membranal sites not directly related to the AChR. A similar mechanism was suggested by Aronstam *et al.* [31] for the non-competitive inhibition of the muscarinic receptor at a high concentration of PCP. In view of the high capacity of the myotubes for PCP, as shown in the present study, such a mechanism cannot be excluded.

Acknowledgements—We wish to thank Mrs. A. Biegelman and Mrs. T. Zinman for their skillful technical assistance and Mrs. B. Lederhendler for typing the manuscript.

REFERENCES

1. R. S. Burns and S. E. Lerner, *Clin. Toxic.* **12**, 463 (1978).
2. R. C. Petersen and R. C. Stillman, *NIDA. Res. Monog. Ser.* **21**, 1 (1978).
3. J. P. Vincent, B. Kartalovsky, P. Geneste, J. M. Kamenka and M. Lazdunski, *Proc. natn. Acad. Sci. U.S.A.* **76**, 4678 (1979).
4. S. R. Zukin and R. S. Zukin, *Proc. natn. Acad. Sci. U.S.A.* **76**, 5372 (1979).
5. J. P. Vincent, J. Vignon, B. Kartalovsky and M. Lazdunski, *Eur. J. Pharmac.* **68**, 73 (1980).
6. J. P. Vincent, J. Vignon, B. Kartalovsky and M. Lazdunski, *Eur. J. Pharmac.* **68**, 79 (1980).
7. S. R. Zukin, M. L. Fitz-Syage, R. Nichtenhauser and R. S. Zukin, *Brain Res.* **258**, 277 (1983).
8. J. P. Vincent, J-N. Bidard, M. Lazdunski, G. Romey, Y. Tourneur and J. Vignon, *Fedn. Proc.* **42**, 2570 (1983).
9. S. Maayani and H. Weinstein, *Life Sci.* **26**, 2001 (1980).
10. M. A. Reis, A. Shainberg, A. Amir and P. Fuchs, *Abstracts Eleventh International Congress Biochemistry, Toronto,* p. 556, No. 09-7-R2. National Research Council, Canada, Ottawa, Canada K1A 0R6 (1979).
11. M. A. Reis, A. Amir, A. Gamliel, S. Zangen, A. Shainberg and P. Fuchs, in *Neurotransmitters and Their Receptors* (Eds. U. Z. Littauer, Y. Dudai, I. Silman, V. I. Teichberg and Z. Vogel), p. 303. John Wiley, Chichester (1980).
12. M. C. Tsai, E. X. Albuquerque, R. S. Aronstam, A. T. Eldefrawi, M. E. Eldefrawi and D. J. Triggle, *Molec. Pharmac.* **18**, 159 (1980).
13. E. X. Albuquerque, M. C. Tsai, R. S. Aronstam, A. T. Eldefrawi and M. E. Eldefrawi, *Molec. Pharmac.* **18**, 167 (1980).
14. Y. Kloog, A. Kalir, O. Buchman and M. Sokolovsky, *Fedn. Eur. Biochem. Soc. Lett.* **109**, 125 (1980).
15. Y. Kloog, A. Gabrielevitz, A. Kalir, D. Balderman and M. Sokolovsky, *Biochem. Pharmac.* **28**, 1447 (1979).
16. T. W. Mittag and S. P. Gross, *Abstract, Seventh Meeting of the International Society of Neurochemistry, Jerusalem, Israel,* Sept. 2–6, p. 486 (1979).
17. E. X. Albuquerque, L. G. Aguayo, J. E. Warnick, R. K. Ickowicz and M. P. Blaustein, *Fedn. Proc.* **42**, 2584 (1983).
18. A. Kalir, H. Edery, Z. Pelah, D. Balderman and G. Porath, *J. med. Chem.* **12**, 473 (1969).
19. V. H. Maddox, E. F. Godefroi and R. F. Parcell, *J. med. Chem.* **8**, 230 (1965).
20. A. Kalir, S. Maayani, M. Rehavi, R. Elkavets, I. Pri-Bar, O. Buchman and M. Sokolovsky, *Eur. J. mednl. Chem.* **13**, 17 (1978).
21. A. Kalir, D. Balderman, M. Torten, O. Buchman and I. Pri-Bar, *J. labelled Comp. Radiopharm.* **13**, 445 (1977).
22. A. Shainberg, S. A. Cohen and P. G. Nelson, *Pflügers Archs.* **361**, 255 (1976).
23. A. Shainberg, G. Yagil and D. Yaffe, *Dev. Biol.* **25**, 1 (1971).
24. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
25. W. A. Catterall, *J. biol. Chem.* **250**, 1776 (1975).
26. M. Reis, *Ph.D. Thesis.* Bar-Ilan University, Ramat-Gan, Israel (1982).
27. L. G. Aguayo, B. Pazhenchevsky, J. W. Daly and E. X. Albuquerque, *Molec. Pharmac.* **20**, 345 (1981).
28. A. Amir, A. Biegelman, A. Kalir and P. Fuchs, *Biochem. Pharmac.* **31**, 1475 (1982).
29. R. Haring, Y. Kloog, A. Kalir and M. Sokolovsky, *Biochem. biophys. Res. Commun.* **113**, 723 (1983).
30. J. W. Karpen, H. Aoshima, L. G. Abood and G. P. Hess, *Proc. natn. Acad. Sci. U.S.A.* **79**, 2509 (1982).
31. R. S. Aronstam, M. E. Eldefrawi, A. T. Eldefrawi, E. X. Albuquerque, K. F. Jim and D. J. Triggle, *Molec. Pharmac.* **18**, 179 (1980).