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SYNERGISTIC INHIBITION BY TRIFLUOPERAZINE AND PHENCYCLIDINE OF CARBAMYLCHOLINE-INDUCED CATION INFLUX IN MUSCLE CULTURES

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Abstract—The effect of trifluoperazine (TFP) and phencyclidine (PCP) on acetylcholine receptor (AChR) function was studied in rat myotubes differentiated *in vitro*. While both drugs exerted an inhibitory effect on carbamylcholine (CCh)-induced Na^+ or Ca^{2+} flux (I_{50} = 5–9 μM), α-bungarotoxin (α-Bgt) binding was not affected. The inhibitory effect of both drugs was independent of CCh concentration. The mutual inhibitory effect of TFP and PCP on Ca^{2+} influx was analyzed using three alternative models of interaction between the two drugs: competitive, additive and synergistic inhibition models. Our results are in accord with a synergistic interaction between the drugs probably not through desensitization. This synergistic interaction between the drugs provides a biochemical rationale to the phenothiazine contraindication in the treatment of PCP psychosis.

Antipsychotic phenothiazines, i.e. TFP‡ and CPZ, were shown to inhibit neuromuscular transmission [1–6]. The mechanism by which phenothiazine exerts this inhibitory effect is under controversy. Several authors suggested a presynaptic site of inhibition [2, 4, 6], while others have pointed to a postsynaptic site [7–11]. It should be noted that most of these studies were performed on preparations which contained both pre- and post-synaptic areas, which might complicate the interpretation of the results. Cultured myotubes which exhibit properties of denervated muscle, are free of nerve cells and therefore provide a suitable model for post-synaptic events occurring at the neuromuscular junction [12, 13]. We have previously used rat muscle cultures to examine the interaction of the psychotrophic drug PCP with nicotinic AChR function, through its inhibition of CCh-induced cation fluxes [14].

Using this system, we have studied the interaction of TFP with the AChR function. Since a potentiating influence of the PCP effects is known to be exerted by phenothiazine during treatment of PCP psychosis (for review [15–17]), it was of interest to analyze their mutual effect on the receptor properties. In the present study we provide biochemical evidence for synergistic inhibition of AChR function by TFP and PCP. These results may explain phenothiazine contraindication in the treatment of PCP psychosis.

EXPERIMENTAL PROCEDURES

Materials. PCP was provided by Dr A. Kalir. Other chemicals were purchased from Sigma. $^{45}CaCl_2$, $^{22}NaCl$ and $Na^{125}I$ were obtained from Amersham. [^{125}I]α-Bgt was prepared by the chloramine T iodination method [18].

Cell cultures. Experiments were carried out on rat myotubes differentiated *in vitro*, 7–10 days after plating. The cells were cultured in collagen-coated 30 mm plastic tissue culture plates (Sterilin), as previously described [19]. A batch of 7–10-day-old myotube cultures was 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.

[^{125}I]α-Bgt binding. The level of the receptors was measured as described previously [18]. Intact muscle cultures were exposed to 6×10^{-8} M [^{125}I]α-Bgt (50–150 Ci/mmole) for 1 hr in the growth medium, or for a shorter period of time (5 min) under the conditions of Ca influx measurements. Non-bound toxin was removed by six washes with 2 ml of phosphate-buffered saline (PBS). Cultures incubated with 1 mM CCh prior and during exposure to [^{125}I]α-Bgt provided the value of non-specific binding (less than 5% of the total binding). Specific binding was obtained by subtracting non-specific from total binding. Bound toxin was measured by laying dishes on a flat crystal 2-in. diameter γ detector (Elscint, Haifa, Israel). Counting efficiency was 28%.

Ion flux measurements. The effect of drugs on CCh-induced $^{45}Ca^{2+}$-influx was measured as follows: The investigated drugs were added to the growth medium and cells were incubated with the drugs at 37° for 30 min. Cultures were then transferred to room temperature, the growth medium was removed, and the cultures were rinsed once and incubated for 1 min with 1 ml of sucrose–histidine buffer (SH-buffer, containing 300 mM sucrose, 30 mM histidine, 5 mM KCl and 2 mM $CaCl_2$, pH 7.4). The medium was replaced with 1 ml of SH-buffer containing 0.2 μCi $^{45}Ca^{2+}$ and influx was initiated by the addition of CCh to a final con-

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‡ Abbreviations used: AChR, acetylcholine receptor; α-Bgt, α-bungarotoxin; CCh, carbamylcholine; CPZ, chloropromazine; PCP, phencyclidine; PBS, phosphate-buffered saline; TFP, trifluoperazine.

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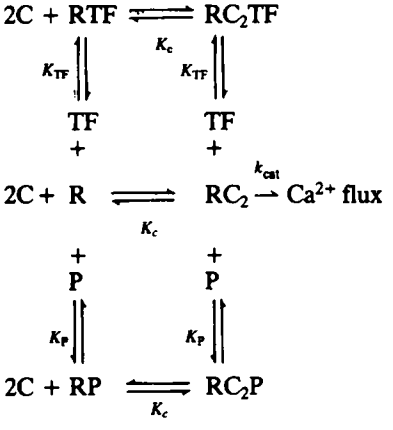
centration of 0.1 mM. After 30 sec, the medium was removed and the cells were washed five times with 1 ml PBS. Cells were then lysed by 0.3 ml of 10% Triton X-100 and collected to counting vials containing 4 ml of scintillation fluid (Hydrofluor, National Diagnostics). The basal uptake in the absence of CCh (less than 5% of the total uptake) was subtracted from the total influx; the net influx value is shown in the Results. Radioactivity was determined in a Kontron scintillation counter (about 85% counting efficiency). CCh-induced ^{22}Na influx was measured by the method of Catterall [12].

Each experiment was carried out on sister cultures in triplicate: variations were within 10%. Variations between different batches of cultures were larger, but trends and proportions of various phenomena were maintained.

THEORY

Competitive inhibition model

For a situation in which PCP and TFP compete in their inhibitory effect on CCh-induced Ca^{2+} flux, while each drug exerts a separate inhibitory effect independent of CCh concentration, the following scheme is presented:



Scheme a.

where C, P and TF stand for CCh, PCP and TFP, respectively; R is the free nicotinic receptor. RC_2, RP, RTF are binary complexes of the receptor with the respective ligands. RC_2P and RC_2TF are ternary complexes. K_c, K_p and K_{TF} are dissociation equilibrium constants of binding of CCh, PCP and TFP, respectively, to their sites of interaction. k_{cat} is the catalytic rate constant of the ion flux.

Here both PCP and TFP are assumed to compete in a mutually exclusive manner on a common site situated on the nicotinic receptor. Both drugs are assumed to compete non-competitively with CCh on the receptor.

The mass conservation equation gives the following expression for the total receptor concentration

$$[R_T] = [R] + [RC_2] + [RP] + [RTF] + [RC_2P] + [RC_2TF] \quad (1)$$

The concentration of RC_2, the active form of the receptor, is thus given by

$$[RC_2] = \frac{[R_T]}{\left(\frac{K_c}{[C]^2} + 1\right)\left(\frac{[P]}{K_P} + \frac{[TF]}{K_{TF}} + 1\right)} \quad (2)$$

Ca^{2+} flux in the presence of both PCP and TFP, $(V_{TF})_P$, is given by the following expression:

$$(V_{TF})_P = \frac{k_{cat}[R_T]}{\left(\frac{K_c}{[C]^2} + 1\right)\left(\frac{[P]}{K_P} + \frac{[TF]}{K_{TF}} + 1\right)} \quad (3)$$

Ca^{2+} flux in the presence of TFP alone, V_{TF}, is given by:

$$V_{TF} = \frac{k_{cat}[R_T]}{\left(\frac{K_c}{[C]^2} + 1\right)\left(\frac{[TF]}{K_{TF}} + 1\right)} \quad (4)$$

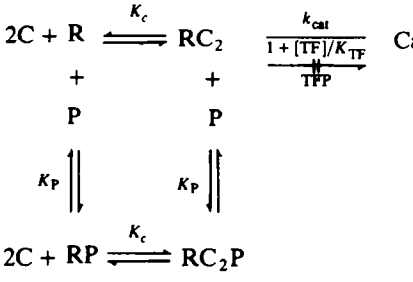
The ratio $(V_{TF})/(V_{TF})_P$ can thus be derived:

$$\frac{V_{TF}}{(V_{TF})_P} = 1 + \frac{[P]}{K_P(1 + [TF]/K_{TF})} \quad (5)$$

This equation predicts that $(V_{TF})/(V_{TF})_P$ versus [P] will, at different TFP concentrations, yield a series of straight lines with an intercept of 1 and a slope, diminishing as a function of [TF], which equals $1/K_P(1 + [TF]/K_{TF})$. A *reciprocal* plot of $1/slope$ as a function of [TF] is expected, according to this model, to give a straight line, with an intercept of K_P and a slope of K_P/K_{TF}.

Additive-inhibition model

To get an additive-inhibition effect of PCP and TFP on CCh-induced Ca^{2+} flux, the drugs should interact with the system at two different non-interacting sites. An inhibition of CCh-induced Ca^{2+} influx by each drug separately, which is independent of CCh concentration is also incorporated into the following additive-inhibition model:



Scheme b.

Here, PCP is assumed to inhibit the nicotinic receptor non-competitively and TFP is assumed to inhibit the system at a step beyond the receptor, thus affecting k_{cat}. TFP inhibition is incorporated quantitatively dividing k_{cat} by $(1 + [TF]/K_{TF})$.

The mass conservation equation for the total nicotinic-receptor concentration, R_T, is given by:

$$[R_T] = [R] + [RP] + [RC_2] + [RC_2P] \quad (6)$$

It can easily be seen that the concentration of the CCh–receptor complex (RC_2) the active form of the receptor, can be expressed by the following equation:

$$[RC_2] = \frac{[R_T]}{(1 + K_c/[C]^2)(1 + [P]/K_P)} \quad (7)$$

Ca^{2+} flux is the product of k_{cat} and $[RC_2]$ and in the presence of both TFP and PCP, Ca^{2+} flux is given by the following expression:

$$(V_{TF})_P = \frac{k_{cat}[R_T]}{(1+[TF]/K_{TF})(1+K_c/[C]^2)(1+[P]/K_P)} \quad (8)$$

while V_{TF} without PCP is:

$$V_{TF} = \frac{k_{cat}[R_T]}{(1 + [TF]/K_{TF})(1 + K_c/[C]^2)} \quad (9)$$

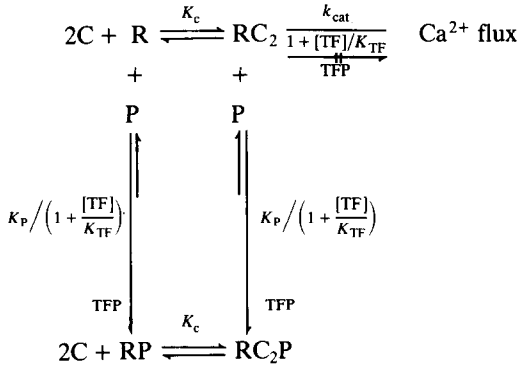
Thus,

$$V_{TF}/(V_{TF})_P = 1 + [P]/K_P. \quad (10)$$

This equation predicts that the ratio $(V_{TF})/(V_{TF})_P$ will be independent of [TFP]. It also predicts that $(V_{TF})/(V_{TF})_P$ versus [P] will yield a straight line with its intercept (= 1) and its slope (= $1/K_P$) both independent of [TFP].

Synergistic-inhibition model

A model describing synergistic-inhibition by PCP and TFP of CCh-induced Ca^{2+} flux should account for a potentiation of PCP effect by TFP, as well as for a [CCh] independent inhibition by each drug separately, of CCh-induced Ca^{2+} flux. Such a model is presented in Scheme c.



Scheme c.

In this model, it is assumed that TFP affects Ca^{2+} flux by interacting with the system at a step beyond the receptor. TFP is also assumed to potentiate PCP effect, increasing its affinity to the receptor. These inhibitory and potentiating effects of TFP are quantitatively entered as denominators into the catalytic constant, k_{cat}, and into the equilibrium dissociation constant of PCP (K_P).

The mass conservation equation for R_T gives

$$[R_T] = [R] + [RP] + [RC_2] + [RC_2P].$$

In the presence of both PCP and TFP, the active form of the receptor, RC_2 is given by the following equation:

$$[RC_2] = \frac{[R_T]}{\left(\frac{K_c}{[C]^2} + 1\right)\left(\frac{[P]([TF]/K_{TF} + 1)}{K_P} + 1\right)} \quad (11)$$

The Ca^{2+} flux is then given by:

$$(V_{TF})_P = \frac{k_{cat}[R_T]}{\left(\frac{[TF]}{K_{TF}} + 1\right)\left(\frac{K_c}{[C]^2} + 1\right)\left(\frac{[P]([TF]/K_{TF} + 1)}{K_P} + 1\right)} \quad (12)$$

In the presence of TFP alone, Ca^{2+} flux is expressed by:

$$V_{TF} = \frac{k_{cat}[R_T]}{\left(\frac{[TF]}{K_{TF}} + 1\right)\left(\frac{K_c}{[C]^2} + 1\right)} \quad (13)$$

The ratio of these fluxes is, then:

$$V_{TF}/(V_{TF})_P = 1 + \frac{[P]([TF]/K_{TF} + 1)}{K_P} \quad (14)$$

This equation predicts, plotting $V_{TF}/(V_{TF})_P$ versus [P], a series of straight lines with an intercept of 1 and a slope which equals

$$\left(\frac{[TF]}{K_{TF}} + 1\right)\Big/ K_P$$

Plotting the slope as a function of [TF] predicts a straight line with an intercept = $1/K_P$ and a slope which equals $1/(K_{TF}K_P)$.

This equation also predicts, plotting $(V_{TF})/(V_{TF})_P$ versus [TF], a series of straight lines. Here, the lines have an intercept = $1 + [P]/K_P$ and a slope which equals $[P]/(K_PK_{TF})$. Plotting the intercept as a function of [P] predicts a straight line with a second intercept = 1 and a second slope which equals $1/K_P$. Plotting the slope as a function of [P] yields another straight line with its second intercept = 0 and its second slope = $1/(K_PK_{TF})$.

RESULTS AND DISCUSSION

Inhibition of CCh*-induced ion fluxes by TFP*

The effect of TFP on CCh-induced ion fluxes and on α-Bgt binding in rat myotubes differentiated *in vitro* are shown in Fig. 1. A dose-dependent inhibition of both $^{22}Na^+$ and $^{45}Ca^{2+}$ uptake was exerted by TFP. The concentration of TFP giving a 50% inhibition (= I_{50}) was 5–9 μM. [^{125}I]α-Bgt binding was not affected by TFP at concentrations up to 100 μM. This result confirms previous studies in other preparations which have also shown that TFP at concentrations up to 100 μM did not react with α-Bgt binding sites [11, 20]. Since similar results were obtained measuring both sodium and calcium uptake, complete quantitative analysis was performed on calcium fluxes. The advantages of measuring Ca^{2+} fluxes are as follows. (a) The energy of $^{45}Ca^{2+}$ irradiation is smaller than that of $^{22}Na^+$ and can be easily attenuated. (b) The K_m value for Ca^{2+}

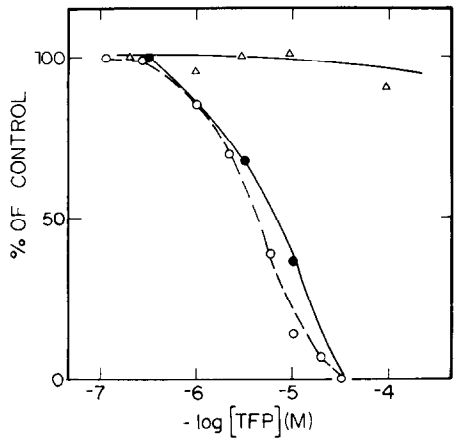


Fig. 1. Effect of TFP on acetylcholine receptor function and on [^{125}I]α-Bgt binding. Acetylcholine receptor function in myotubes grown in 30 mm dishes was measured by $^{22}Na^+$ and $^{45}Ca^{2+}$ uptake in response to 0.1 mM CCh. CCh-induced Ca^{2+} uptake (100%) was 36 nmole/30 sec/dish. CCh-induced Na^+ uptake; 100% value was 1.16 μmole/30 sec/dish. α-Bgt (100%) was 0.560 pmole/dish. Key: Δ, α-Bgt binding; $\circ$, Ca^{2+} uptake; and $\bullet$, Na^+ uptake.

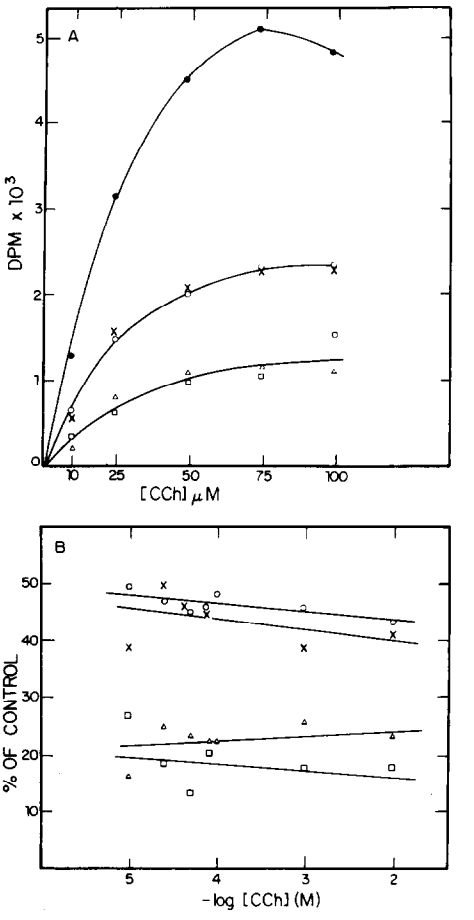


Fig. 2. (A) Dose–response curve for CCh-induced Ca^{2+} uptake in the presence of TFP or PCP. AChR function was measured by Ca^{2+} uptake in response to various concentrations of CCh in the presence of either TFP or PCP. Key: $\bullet$, control without PCP and TFP; $\circ$, 5 μM TFP; $\square$, 10 μM TFP; $\times$, 5 μM PCP; and Δ, 10 μM PCP. (B) Analysis of results shown in (A). The ratio between Ca^{2+} flux in the presence of TFP or PCP and the control flux is shown as a function of CCh concentration.

uptake through the receptor is 0.4 mM (unpublished results), more than 200 times lower than the K_m value for Na^+ uptake [12], making Ca^{2+} uptake studies, at 2 mM of Ca^{2+}, safer and more accurate. Moreover, the low K_m for Ca^{2+} influx enables us to perform our studies using saturation Ca^{2+} concentrations, studies which are not possible using Na^+ influx. (c) Na^+ influx studies should be performed in the presence of ouabain which inhibits the Na^+ pump. This drug, which changes membrane potential [21] and thus influences ion fluxes [22], is not needed when working on Ca^{2+} influx, since Ca^{2+} efflux at room temperature is negligible in comparison to Ca^{2+} uptake by the sarcoplasmic reticulum [23]. Furthermore, Ca^{2+} efflux from the myotube cultures was shown to be dependent on extracellular Na^+ [24]. Since Na was eliminated from the uptake medium, no activity of Na/Ca exchanger is expected. (d) Depolarization induced by high K^+ did not elevate Ca^{2+} influx into the myotubes (data not shown). This finding eliminates the possibility that CCh induced Ca^{2+} influx is through secondarily activated sarcolemmal voltage dependent Ca channels.

To elucidate the mechanism by which TFP exerts its inhibitory effect on receptor function, dose–response curves of CCh-induced Ca^{2+} influx in the presence and absence of TFP were performed (Fig. 2A). Inhibition of the AChR function by TFP was independent of CCh concentration (Fig. 2B). It was suggested that TFP enhanced desensitization of the nicotinic receptor [11]. Such a mechanism should predict a greater inhibitory effect of TFP at higher CCh concentrations. However, our results (Fig. 2) are not consistent with such a mechanism. Moreover, the fact that TFP does not affect α-Bgt binding, and that TFP inhibition is CCh-independent, also excludes the possibility of competitive interaction between CCh and TFP. Therefore, we have included in our model (see Theory Section) a non-competitive manner of TFP-inhibition at, or beyond, the receptor site.

Inhibition of CCh*-induced* Ca^{2+} *uptake by PCP*

We have previously shown an inhibition of CCh-induced ion fluxes by PCP and its analogs in muscle cultures [14]. It was also shown that PCP did not inhibit α-Bgt binding [14]. In the Torpedo electric organ membrane a non-competitive interaction of PCP with a site distinct from the acetylcholine binding site was suggested [25–27]. To verify such an interaction in our system, dose–response curves of CCh-induced Ca^{2+} fluxes in the presence and absence of PCP were conducted. Figure 2 shows that PCP inhibition is independent of CCh concentrations. These results are consistent with a non-competitive pattern of inhibition of CCh-induced receptor function by PCP. This type of PCP-inhibition was therefore included in our model (see Theory Section).

Table 1. Inhibition of CCh-induced Ca^{2+} influx by TFP and PCP

TFP (μM)	PCP (μM)				
	0	1	3	5	7
0	100	100	85	67	59
1	99	—	70	53	38
3	92	69	49	44	34
5	76	57	40	33	25
7	78	53	39	29	19
9	54	27	23	17	—

Eight-day-old rat muscle cultures received either TFP or PCP alone, or a combination of the two drugs at various concentrations, as indicated. CCh-induced Ca^{2+} uptake in the absence of both inhibitors is taken as 100% control uptake. The data shown are presented as the ratio between the CCh-induced Ca^{2+} influx of the inhibitor-treated myotubes and the control. Values are average of three replicates of a representative experiment. Variation among the triplicates was less than 10%. 100% CCh-induced Ca^{2+} uptake was 26 nmoles/30 sec/dish.

Mutual inhibition of CCh-*induced* Ca^{2+} *influx by both TFP and PCP*

Controversy exists with regard to the use of medication in the treatment of both the severe agitation-excitement and the concomitant psychotic symptoms frequently associated with PCP intoxication and poisoning. Luisada and Brown [28] recommended the use of CPZ, whereas Domino [29] reported that when CPZ was combined with PCP, there was a marked potentiation of the depressant action of PCP. The use of a non-phenothiazine agent such as haloperidol, when anti-psychotic medication is indicated in PCP psychosis, was also suggested by others [16]. Further evidence that the CPZ did not antagonize the effect of PCP was provided by Blaster and Chait [17].

Our muscle culture system enables us to analyse quantitatively, on a biochemical level, the combined effect of the phenothazine drug, TFP, and PCP on nicotinic-receptor function. The effect of the simultaneous presence at different concentrations of both TFP and PCP on CCh-induced Ca^{2+} influx was measured (Table 1). It is obvious that the presence of both drugs induced an inhibitory effect which was greater than the inhibition of each drug alone. The three possible general mechanisms which can be consistent with such a pattern of interaction, i.e. competitive, additive and synergistic inhibition, are presented and mathematically analysed in the Theory Section.

Analysis of the mutual inhibitory effect of PCP and TFP

Analysis of the results shown in Table 1 was performed using the definitions presented in the Theory Section. Figure 3A introduces the ratio between Ca^{2+} influx in the presence of TFP alone, V_{TF}, and Ca^{2+} influx in the presence of both TFP and PCP, $(V_{TF})_P$, as a function of PCP concentration. This presentation yields a series of straight lines with positive slopes increasing linearly with TFP concentration (Fig. 3B). The lines intersect the ordinate at the same point independent of TFP concentration, giving an intercept value of one (Fig. 3B). Similar representation of the ratio of Ca^{2+} influxes, $(V_{TF})/(V_{TF})_P$, as a function of TFP (Fig. 4A), also yields a series of straight lines with positive slopes increasing linearly with PCP concentration (Fig. 4B). The intercept of these lines also increases linearly with PCP concentration (Fig. 4B).

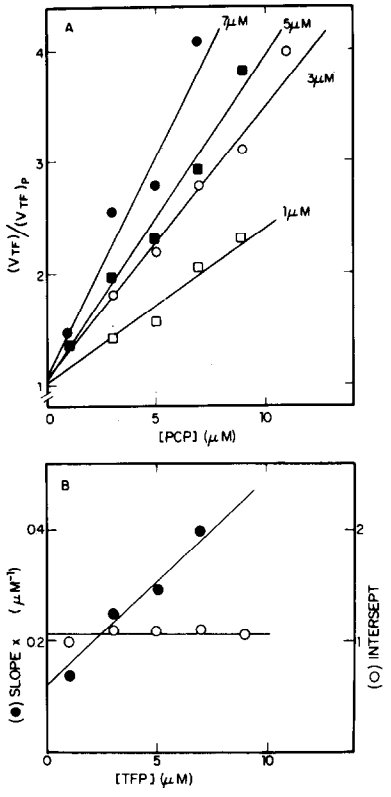


Fig. 3. Analysis of the combined inhibitory effect of PCP and TFP on CCh-induced Ca^{2+} influx. (A) The ratio between Ca^{2+} influx in the presence of TFP alone, V_{TF}, and Ca^{2+} influx in the presence of both TFP and PCP, $(V_{TF})_P$, is presented as a function of PCP concentration. Each straight line represents experiments performed at the same TFP concentration which is specified in the figure. (B) The slopes and intercepts of the straight lines shown in (A) are represented as a function of TFP concentration.

The result of an increasing slope of the lines in Fig. 3A as a function of TFP (Fig. 3B) contradicts the predictions of the competitive-inhibition model. According to this model, these lines should have a diminishing slope as a function of TFP (equation 5), rather than an increasing one. This model is also contradicted by the results presented in Fig. 4. The lines shown in this figure should have a negative, rather than a positive slope according to the competitive model (equation 5).

According to the additive-inhibition model, the ratio $(V_{TF})/(V_{TF})_P$ should be independent of TFP concentration (equation 10). This is not the case in our study (Fig. 4). Furthermore, this model also predicts that the slopes of the lines in Fig. 3A should be independent of TFP concentration. However, our

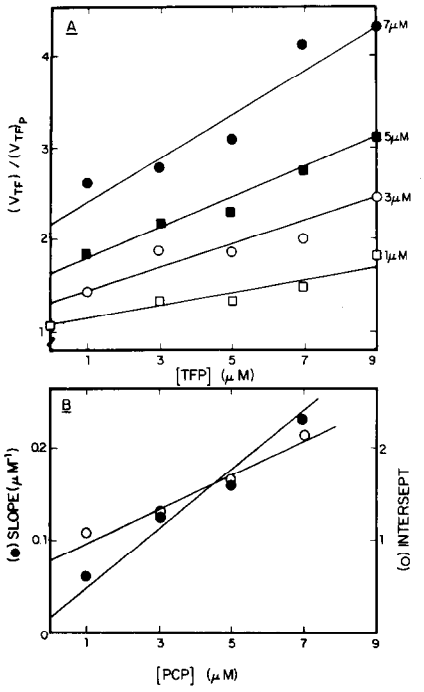


Fig. 4. Analysis of the combined inhibitory effect of PCP and TFP on CCh-induced Ca^{2+} influx. (A) The ratio between Ca^{2+} influx in the presence of TFP alone, V_{TF}, and Ca^{2+} influx in the presence of both TFP and PCP, $(V_{TF})_P$, is presented as a function of TFP concentration. Each straight line represents experiments performed at the same TFP concentration which is specified in the figure. (B) The slopes and intercepts of the straight lines shown in (A) are represented as a function of PCP concentration.

results are not in agreement with this prediction (Fig. 3B).

The synergistic-inhibition model fits well with the experimental results. Equation 14 developed according to this model (see Theory Section) predicts that plotting $(V_{TF})/(V_{TF})_P$ as a function of [PCP] will give a series of straight lines. These lines should have a common intercept at $(V_{TF})/(V_{TF})_P = 1$ and a slope which should be linearly dependent on [TFP]. The results of this study confirm these predictions (Fig. 3). The value of the intercept of the straight line given in Fig. 3B should, according to equation 14, equal $1/K_P$, while the slope of this line should be given by $1/(K_P K_{TF})$. Thus, we can determine experimentally the dissociation constants of both PCP and TFP from their sites of interaction: $K_P = 8.3 \times 10^{-6}$ M and $K_{TF} = 2.8 \times 10^{-6}$ M.

The synergistic-inhibition model similarly predicts that $(V_{TF})/(V_{TF})_P$ as a function of [TFP] will yield a series of straight lines. Both the intercept and the slope of these lines should be linearly dependent on [PCP]. Our results confirm this prediction (Fig. 4). According to equation 14, the second intercept of the intercept versus [PCP] plot should equal 1, which is truly the case (Fig. 4B). The slope of this line should be given by $1/K_P$.

The slope versus [PCP] plot should intersect the origin (equation 14). This is really the case in our study (Fig. 4B). The second slope of the slope versus [PCP] plot should equal $1/(K_P K_{TF})$ (equation 14). Calculating the two dissociation equilibrium constants from this way of presentation, one gets $K_P = 5.3 \times 10^{-6}$ M and $K_{TF} = 5.7 \times 10^{-6}$ M. These values are essentially similar to those calculated from the first way of presentation, lending further support to the predictions of the synergistic-inhibition model.

The value for the dissociation equilibrium constant of PCP (K_P), obtained in our study is in agreement with those obtained for this constant in other studies [15, 27].

We have shown in this study that the TFP site of interaction in our system is different from both CCh- and PCP-binding sites. TFP is known to interact with the Ca^{2+}-binding protein, calmodulin, which triggers several Ca^{2+}-dependent enzymatic activities [30, 31]. We cannot rule out the possibility that TFP inhibitory effects in our system are calmodulin-dependent. However, considering the facts that both CCh-induced Ca^{2+} influx and TFP inhibition of this influx are quite similar at high and low temperature (4° vs 37°, data not shown), it is unlikely that calmodulin-dependent enzymatic activities are involved in the activation of the receptor function. Indeed, recent investigations have shown that TFP is not a specific inhibitor of calmodulin-dependent enzymes [32, 33].

Our results of a synergistic interaction between TFP and PCP on the inhibition of receptor function are in agreement with previously reported potentiating influences of TFP on PCP side effects in patients with PCP psychosis [17], thus providing a biochemical rationale to the phenothiazine contraindication in the treatment of PCP psychosis.

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