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Effects of acute and chronic treatment on the pro- and anti-convulsant actions of CL 218, 872, PK 8165 and PK 9084, putative ligands for the benzodiazepine receptor

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CL 218,872 is a triazolopyridazine that acts at the benzodiazepine binding site. At low doses (0·5–7·5 mg kg^{-1}) it is proconvulsant when combined with subconvulsant doses of picrotoxin but not when combined with pentetrazol (leptazol, pentylenetetrazol). At high doses (20–60 mg kg^{-1}) CL 218,872 counteracted seizures caused by pentetrazol but not those caused by picrotoxin. There was tolerance to the proconvulsant effects after five days of treatment and to the anticonvulsant effects after 15–20 days. Two phenylquinolines, PK 8165 and PK 9084, that also act at the GABA-benzodiazepine receptor complex have proconvulsant actions in combination with picrotoxin. Significant tolerance to these effects had not developed even after 20 days of treatment. It is concluded that three different sites on the GABA-benzodiazepine complex mediate the pro- and anti-convulsant actions of CL 218,872 and the proconvulsant actions of PK 8165 and PK 9084.

The phenylquinolines PK 8165 and PK 9084, and the triazolopyridazine CL 218,872 (3-methyl-6[3-(trifluoromethyl)phenyl]-1,2,4-triazolo [4,3-6]-pyridizine) are putative novel anxiolytic drugs, thought to act at the benzodiazepine receptor. CL 218,872 displaces benzodiazepines from their binding sites (Lippa et al 1979), like the benzodiazepines, it is both sedative (Straughan et al 1982; File et al 1985) and anxiolytic in the punished drinking test (Lippa et al 1979) and the social interaction test (File 1982). More recently, it has been reported that CL 218,872 has some benzodiazepine antagonist properties. It antagonizes the diazepam-induced loss of righting reflex and increases the dose of diazepam required to protect against bicuculline-induced seizures. It has thus been proposed that CL 218,872 may be a partial agonist at the benzodiazepine receptor (Gee et al 1983a, b).

The phenylquinolines displace benzodiazepines from their binding sites, in-vitro (LeFur et al 1981). They produce a dose-related decrease in locomotor activity, rearing and exploratory head-dipping in the holeboard (File 1983), and therefore may be sedative. The evidence for their anxiolytic action is weak. They increase punished drinking in the rat (Le Fur et al 1981), but unpunished drinking is also increased (Pellow 1985). PK 8165 is not anxiolytic in the social interaction test and PK 9084 is only partially anxiolytic in this test (File & Lister 1983), and in three other animal tests they have no anxiolytic action (Keane et al 1984).

These three drugs differ markedly from the benzodiazepines in their effects on chemically-induced seizures. A recent investigation carried out by Melchior et al (1984) into the action of CL 218,872 on seizures induced by bicuculline, pentetrazol and picrotoxin revealed that high doses of CL 218,872 were anticonvulsant against pentetrazol-induced seizures whereas low doses of CL 218,872 (0·5 mg kg^{-1}) were proconvulsant with subconvulsant doses of picrotoxin or bicuculline, although not with subconvulsant doses of pentetrazol. The phenylquinolines have been shown to antagonize the anticonvulsant effect of diazepam against bicuculline-induced seizures (Gee et al 1983c) and are proconvulsant with subconvulsant doses of picrotoxin and pentetrazol (File & Simmonds 1984).

Experiment 1 was designed to investigate further the proconvulsant and anticonvulsant actions of CL 218,872 with the chemical convulsants picrotoxin and pentetrazol. The aim of experiment 2a was to investigate the effect of chronic treatment with CL 218,872 on the proconvulsant and anticonvulsant actions of this drug. The aim of experiment 2b was to investigate the effect of chronic treatment with the phenylquinolines PK 8165 and PK 9084 on the

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proconvulsant actions of these drugs when combined with a subconvulsant dose of picrotoxin.

METHODS

Animals

Male albino mice, Tuck No. 1 strain (Tuck and Sons, Battlesbridge), 30–40 g, were used in experiments 1 and 2b and male albino T.O. mice (Bantin and Kingman), 25–30 g at the start of chronic treatment, were used in experiment 2a. The mice were housed in groups of eight in a room with a 12 h light: 12 h dark cycle (lights on at 07 00 h) and were allowed free access to food and water.

Drugs

CL 218,872, PK 9084 and PK 8165 were suspended in distilled water with a drop of Tween (polysorbate) 20. Pentetrazol and picrotoxin were both dissolved in distilled water. Control animals received a water/Tween control as appropriate. All drugs were made up to an injectable volume of 4 ml kg^{-1}. CL 218,872 was injected orally 1 h before testing, PK 9084 and 8165 30 min before testing and pentetrazol and picrotoxin immediately before testing. Picrotoxin, pentetrazol, PK 9084 and PK 8165 were administered intraperitoneally.

Treatment

Eight mice were randomly allocated to each drug group. The treatment groups for experiment 1 are shown below and the treatment groups for experiments 2a and 2b are shown in Table 1. In experiments 2a and 2b all animals received 20 days' chronic treatment with either the appropriate drug or a water/Tween 20 control.

Experiment 1

Picrotoxin 3 mg kg^{-1} alone or with CL 218,872 (0·5, 2·5, 5·0, 7·5, 10·0 mg kg^{-1}).

Pentetrazol 30 mg kg^{-1} alone or with CL 218,872 (0·5, 2·5, 5·0, 7·5, 10·0 mg kg^{-1}).

Picrotoxin 8 mg kg^{-1} alone or with CL 218,872 (20, 30, 40, 60 mg kg^{-1}).

Pentetrazol 80 mg kg^{-1} alone or with CL 218,872 (20, 30, 40, 60 mg kg^{-1}).

Procedure

The convulsant and proconvulsant doses of pentetrazol and picrotoxin respectively were selected for each strain of mouse by pilot studies. Pilot studies were also carried out to establish the proconvulsant doses of the phenylquinolines.

In experiment 1, the animals received no chronic drug treatment. In experiment 2a all groups received 20 days' oral treatment with either CL 218,872 or water/Tween control, as appropriate. In experiment 2b all groups received 20 days' pretreatment with a water/Tween 20 control, PK 9084 or PK 8165 administered intraperitoneally.

Table 1. The drug pretreatment received by the mice, its duration and the drugs given on the test day in experiments 2a and 2b. All doses are in mg kg^{-1}. (CL = CL 218,872, Picro = picrotoxin, PTZ = pentetrazol).

Pretreatment	Time (days)	Test day
Experiment 2a		
Vehicle	20	Picro 2
		Picro 2 + CL 2·5
		PTZ 80
		PTZ 80 + CL 20
CL 2·5 mg kg^{-1}	5	Picro 2 + CL 2·5
	15	Picro 2 + CL 2·5
	20	Picro 2 + CL 2·5
CL 20 mg kg^{-1}	5	PTZ 80 + CL 20
	15	PTZ 80 + CL 20
	20	PTZ 80 + CL 20
Experiment 2b		
Vehicle	20	Picro 3
		Picro 3 + PK 9084 5
		Picro 3 + PK 8165 5
PK 9084 5* mg kg^{-1}	5	Picro 3 + PK 9084 5
	10	Picro 3 + PK 9084 5
	20	Picro 3 + PK 9084 5
PK 8165 5† mg kg^{-1}	5	Picro 3 + PK 8165 5
	10	Picro 3 + PK 8165 5
	20	Picro 3 + PK 8165 5

* 2-Phenyl-4-[2-(4-piperidinyl)ethyl]quinoline.
† 2-Phenyl-4-[2-(4-piperidinyl)ethyl]quinidine.

In all these experiments the mice were tested in subdued lighting between 14 00 and 19 00 h. In experiments 1 and 2a the animals received one injection of CL 218,872 or water/Tween 20 administered orally one hour before the test and one intraperitoneal injection of pentetrazol or picrotoxin as appropriate just before testing. In experiment 2b the animals received one injection of water/Tween control, PK 9084 or PK 8165 intraperitoneally 30 min before the test and one injection of picrotoxin intraperitoneally immediately before testing.

Immediately after this second injection the mice were placed, individually, in transparent sided cages and were observed for 30 min. Latencies to the first myoclonic spasm and the first seizure were recorded, and the number of animals in each treatment group of eight which had myoclonic spasms or full seizures by the end of the test period was also recorded. A myoclonic spasm was defined as a sudden extension of the fore- or hind-limbs and a full seizure as repeated tonic-clonic extensions and contractions of both fore- and hind-limbs.

Statistics

The mean latencies to first myoclonic spasm and first seizure for each group were compared with the control values by means of independent *t*-tests. The numbers of animals in each group that had seizures or spasms were compared with control values by the Fisher exact probability test.

RESULTS

Experiment 1: Acute proconvulsant and anticonvulsant effects of CL 218,872

(a) Proconvulsant effects.

When a subconvulsant dose of picrotoxin (3 mg kg^{-1}) was administered to the mice alone, no myoclonic spasms or seizures were observed. However, in the presence of CL 218,872 a significant increase was observed in the number of animals that had spasms in the 0·5, 2·5, 5·0 and 7·5 mg kg^{-1} dosage groups ($P < 0·01$ in each case). Some full seizures were also observed at the 2·5 and 5·0 mg kg^{-1} doses of CL 218,872 in the presence of picrotoxin (3 mg kg^{-1}). No myoclonic spasms or seizures were observed in any animals when pentetrazol (30 mg kg^{-1}) was administered alone and no myoclonic spasms or seizures were observed in any of the groups when this dose of pentetrazole was tested with CL 218,872 at 0·5, 2·5, 5·0 and 7·5 mg kg^{-1} (Table 2).

(b) Anticonvulsant effects.

When pentetrazol at 80 mg kg^{-1} was tested alone, myoclonic spasms and seizures were observed in all the animals in the group. CL 218,872 at 20, 30, 40 and 60 mg kg^{-1} did not cause a significant decrease in the number of animals that had myoclonic spasms, however a significant increase in the mean latency to the first myoclonic spasm was observed at all these doses ($P < 0·05$). No seizures were observed in any of those groups which received CL 218,872 at 20, 30, 40 and 60 mg kg^{-1} with pentetrazole at 80 mg kg^{-1}. Picrotoxin at 8 mg kg^{-1} tested alone caused myoclonic spasms and seizures in all the animals in the test group. When CL 218,872 was tested at 20, 30, and 40 mg kg^{-1}, no decrease in the number of animals in each group that had myoclonic spasms and seizures was observed. However, significant increases were observed in the mean latencies to the first spasm in the CL 218,872 20 and 30 mg kg^{-1} groups and in the mean latencies to the first seizure in all the groups tested ($P < 0·05$ in all cases) (Table 3).

Table 2. The proconvulsant effect of acute doses of CL 218,872. The subconvulsant doses of picrotoxin and pentetrazol (PTZ) used were 3 mg and 30 mg kg^{-1} respectively. The numbers of animals in each group which had myoclonic spasms or full seizures are shown and mean latency(s) to the first myoclonic spasm (±s.e.m.) for each group. All doses are in mg kg^{-1}.

Proconvulsant effect Drug treatment	No of myoclonus	No of seizures	Latency to myoclonus
PTZ 30 alone	0/8	0/8	—
PTZ 30 + CL 0·5	0/8	0/8	—
PTZ 30 + CL 2·5	0/8	0/8	—
PTZ 30 + CL 5	0/8	0/8	—
PTZ 30 + CL 7·5	0/8	0/8	—
Picro 3 alone	0/8	0/8	—
Picro 3 + CL 0·5	5/8*	0/8	880·0 ± 10·0
Picro 3 + CL 2·5	7/8*	2/8	1147·9 ± 33·9
Picro 3 + CL 5	8/8*	3/8	881·9 ± 16·9
Picro 3 + CL 7·5	5/8*	0/8	1052·0 ± 46·6

* Significantly different from control ($P < 0·01$).

Table 3. The anticonvulsant effects of acute doses of CL 218,872. The anticonvulsant doses of picrotoxin and pentetrazol used were 8 mg kg^{-1} and 80 mg kg^{-1} respectively. The numbers of animals in each group which had myoclonic spasms or full seizures are shown and the mean latency(s) to the first myoclonic spasm (+s.e.m.) for each group. All doses are in mg kg^{-1}.

Anticonvulsant effect Drug treatment	No of myoclonus	No of seizures	Latency to myoclonus
PTZ 80 alone	8/8	8/8	48·1 ± 2·1
PTZ 80 + CL 20	8/8	0/8*	83·1 ± 3·0#
PTZ 80 + CL 30	8/8	0/8*	187·5 ± 14·9#
PTZ 80 + CL 40	8/8	0/8*	147·5 ± 7·4#
PTZ 80 + CL 60	8/8	0/8*	113·1 ± 8·9#
Picro 8 alone	8/8	8/8	323·8 ± 16·0
Picro 8 + CL 20	8/8	8/8	419·4 ± 19·8#
Picro 8 + CL 30	8/8	8/8	421·3 ± 24·4#
Picro 8 + CL 40	8/8	8/8	378·1 ± 35·2

* Significantly different from control ($P < 0·01$).
\# Significantly different from appropriate control ($P < 0·05$).

Experiment 2a: The effect of chronic treatment with CL 218,872

When treated with picrotoxin at 2 mg kg^{-1}, only one animal had a myoclonic spasm and no seizures were observed in any of the animals tested, however, in the presence of CL 218,872 at 2·5 mg kg^{-1} a significant increase was observed in the number of animals which had myoclonic spasms ($P = 0·02$). Chronic treatment with CL 218,872 for 5, 15 and 20 days resulted in the loss of this effect in all cases (see Table 4, Fig. 1).

All the mice in the group treated with pentetrazol

at 80 mg kg^{-1} had myoclonic spasms and seizures. The number of animals having seizures was significantly reduced by CL 218,872 at 20 mg kg^{-1} administered acutely ($P < 0{\cdot}005$), no reduction was observed in the number of animals which had myoclonic spasms. A significant reduction in the number of animals seizing was still present after five days of chronic treatment ($P < 0{\cdot}01$). The group which received five days of chronic treatment also had a significant increase in the mean latency to the first myoclonic spasm ($P < 0{\cdot}05$) compared with the control group. After 15 and 20 days of pretreatment the number of animals seizing with pentetrazol at 80 mg kg^{-1} was still reduced, although this effect was no longer significantly different from the control value (see Table 4).

Experiment 2b: The effects of chronic treatment with PK 8165 or PK 9084

The proconvulsant effects of PK 9084 and PK 8165 persist with chronic treatment both in the case of myoclonic spasms and full convulsions. Some tolerance to the proconvulsant effect does, however, seem to be developing with time for both drugs. A steady and progressive decrease in the number of animals experiencing both myoclonic spasms and seizures was observed with chronic treatment, but this was not significantly different from the acutely treated group even after 20 days of chronic treatment with these drugs (see Fig. 1). In contrast to this proconvulsant effect, shown by an increase in the number of animals experiencing myoclonic spasms, PK 8165 caused an increase in the mean latency to the first myoclonic spasm after chronic treatment for 5, 10 or 20 days ($P < 0{\cdot}05$). Similar increases were not observed for PK 9084.

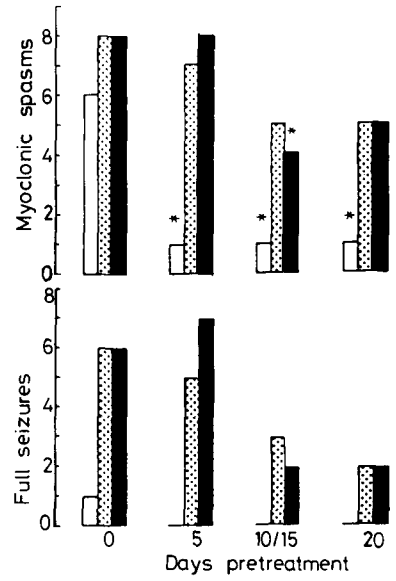

FIG. 1. Number of mice (n = 8) showing myoclonic spasms or full seizures after acute treatment (0 pretreatment days) with CL 218,872 (2·5 mg kg^{-1}) (open columns), PK 8165 (5 mg kg^{-1}) (solid columns) or PK 9084 (5 mg kg^{-1}) (dotted columns), or after 5, 10, 15 or 20 days of daily drug injections.

Table 4. The effects of chronic treatment with CL 218,872 on its pro- and anticonvulsant effects. The subconvulsant dose of picrotoxin used was 2 mg kg^{-1} and the convulsant dose of pentetrazol 8 mg kg^{-1}. The numbers of animals in each group which had myoclonic spasms or full seizures are shown and the mean latency(s) to the first myoclonic spasm (±s.e.m.) for each group.

Pretreatment	Test	No of myoclonus	No of seizures	Latency to myoclonus
		Proconvulsant effect		
Vehicle	Pic 2 alone	1/8	0/8	—
Vehicle	Picro 2 + CL 2·5	6/8*	1/8	755·8 ± 102·1
CL 2·5: 5 days	Picro 2 + CL 2·5	1/8	0/8	—
CL 2·5: 15 days	Picro 2 + CL 2·5	1/8	0/8	—
CL 2·5: 20 days	Picro 2 + CL 2·5	1/8	0/8	—
		Anticonvulsant effect		
Vehicle	PTZ 80 alone	8/8	8/8	82·5 ± 29·9
Vehicle	PTZ 80 + CL 20	8/8	2/8*	161·9 ± 41·8
CL 20: 5 days	PTZ 80 + CL 20	8/8	3/8*	151·9 ± 11·5#
CL 20: 15 days	PTZ 80 + CL 20	8/8	5/8	106·4 ± 27·1
CL 20: 20 days	PTZ 80 + CL 20	8/8	5/8	84·4 ± 9·4

* Significantly different from appropriate control (Picro 2 or PTZ 80 alone) $P < 0{\cdot}04$.
\# Significantly different from control ($P < 0{\cdot}05$).

DISCUSSION

The results of experiment 1 both confirm and expand those of Melchior et al (1984). At low doses (0·5–7·5 mg kg^{-1}) CL 218,872 has a proconvulsant effect, causing both myoclonic spasms and seizures when challenged with a subconvulsant dose of picrotoxin. No such proconvulsant effect was seen when a subconvulsant dose of pentetrazol was used. The doses of CL 218,872 which we found to be most effective in producing the proconvulsant effect were 2·5 and 5·0 mg kg^{-1}. These are a little higher than the proconvulsant dose found by Melchior et al (1984), however this is probably due to a strain difference in the types of mice used. At higher doses (20–60 mg kg^{-1}) CL 218,872 had an anticonvulsant effect against pentetrazol-induced seizures. No such anticonvulsant effect was observed against picrotoxin-induced seizures.

These results demonstrate that it is not simply a question of dose that determines whether CL 218,872 is pro- or anticonvulsant (as would be expected for a partial agonist for the benzodiazepine site). The pro- and anticonvulsant effects of this drug also depend on the type and dose of convulsant used. The rates at which tolerance develops to these two effects are very different and would suggest two different sites of action. Although the high dose effects are benzodiazepine-like we would conclude that CL 218,872 cannot be considered a pure benzodiazepine agonist or even as a partial agonist.

Tolerance to the proconvulsant effect of PK 8165 and PK 9084 when combined with a subconvulsant dose of picrotoxin, developed much more slowly than tolerance to the proconvulsant effects of CL 218,872. This slower time course of development of tolerance suggests that the proconvulsant actions of PK 8165 and PK 9084 may well be mediated by different sites of action and/or mechanisms from the proconvulsant actions of CL 218,872. It has been demonstrated by Keane et al (1984) that, in the case of PK 9084 and PK 8165, although these drugs bind to the benzodiazepine receptor in-vitro, [^{3}H]-flunitrazepam is not displaced by these drugs in-vivo. Therefore although it seems that these compounds act somewhere on the GABA-benzodiazepine receptor complex (File & Simmonds 1984) they do not act directly on the benzodiazepine binding site.

The results from the present studies suggest that there are several sites on the GABA-benzodiazepine receptor complex that can mediate pro- and anticonvulsant drug effects. However to further characterize these sites more biochemical and electrophysiological evidence is required.

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Comparison of β-adrenoceptor populations in cat and guinea-pig left atria

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The subtypes of β-adrenoceptor present in left atrial preparations from the guinea-pig and cat have been assessed using both responses obtained in organ bath experiments and radioligand binding studies. From the positive inotropic responses to procaterol, the pK_B values for practolol using a variety of agonists, and from displacement of [^{125}I]cyanopindolol from left atrial membrane homogenates by the selective β_1- and β_2-adrenoceptor antagonists L643,717-01J10 and ICI 118,551, it was concluded that guinea-pig left atria possess only β_1-adrenoceptors, whilst cat left atria possess both β_1- and β_2-adrenoceptor subtypes.

Positive inotropic responses to sympathomimetic drugs in left atrial preparations result from activation of β-adrenoceptors located throughout the tissue, and one atrium yields a sufficient quantity of membrane homogenate for receptor binding experiments with [^{125}I]cyanopindolol. Thus, in general, left atrial preparations are well-suited for correlating organ bath responses with results from radio ligand binding studies.

Previous studies have indicated that inotropic responses in both guinea-pig and cat left atria result from the activation of homogeneous populations of β_1-adrenoceptors within the tissues (Vlietstra & Blinks 1976; Kaumann et al 1978; Zaagsma et al 1979; McPherson et al 1984). However, more recent studies utilizing the highly selective β_2-adrenoceptor agonist procaterol, have led to the suggestion that both β_1- and β_2-adrenoceptors may be involved in the inotropic actions in the two species (Johansson & Persson 1983; Kaumann et al 1983).

In the present study we have sought to clarify the divergent results utilizing both organ bath experiments in which inotropic activity in left atrial preparations from guinea-pigs and cats has been assessed, and radioligand binding studies using membrane preparations of the same tissues.

METHODS

Left atria were taken from reserpine-pretreated guinea-pigs (1 mg kg^{-1}, i.p. 18 h, 200–500 g) and α-chloralose (80 mg kg^{-1}, i.p.) anaesthetized cats (0·25 mg kg^{-1}, i.p. 18 h, 400–1000 g: age >8 weeks). Pretreatment of both species with reserpine insured that responses obtained to exogenous agonists and their interaction with selected antagonists, were not complicated by concurrent release of endogenous neurotransmitter from sympathetic nerve terminals. Preparations, which were bathed at 37 °C in Krebs solution (NaCl 118, KCl 4·7, $CaCl_2$ 1·9, $NaHCO_3$ 25, $MgSO_4$ 1·2, glucose 11·7, NaH_2PO_4 1·2; EDTA 0·1, ascorbic acid 0·1 mM) gassed with 5% CO_2 in O_2, were electrically driven at 2·5 Hz using pulses of 1 ms duration at twice threshold driving voltage (range 1–3 V). Neuronal and extraneuronal uptake were inhibited by either pretreating tissues with phenoxybenzamine (50 μM, 30 min incubation, followed by 6 washes in 30 min) or by the addition of desipramine (1 μM) and hydrocortisone (50 μM) to the bathing solution. Changes in tension were recorded using a Grass FT03c transducer coupled to a Grass polygraph. In the experiments in which the effects of procaterol were studied, constant cumulative concentration-effect curves were first established to (−)-isoprenaline followed by a curve for procaterol.

In other experiments, concentration-effect curves were established to (−)-noradrenaline, (−)-adrenaline, (−)-isoprenaline and (±)-*N*-*t*-butylnoradrenaline before and after a 30 min incubation period with practolol (5 μM). Agonist EC50 values in the absence and presence of antagonists were used to calculate pK_B values ($-\log K_B$, Furchgott 1972).

In radioligand binding studies (see McPherson et al 1984 for full description), left atria were taken from non-reserpine-pretreated guinea-pigs and cats, and homogenized in ice-cold Krebs phosphate buffer (NaCl 119, KCl 4·8, $MgSO_4$ 1·2, $CaCl_2$ 1·9, glucose 11·7, NaH_2PO_4 1·3, Na_2HPO_4 8·7 mM, pH 7·4). Following centrifugation, membrane pellets were resuspended in 300 vol. buffer. Triplicate assays were performed in disposable polystyrene tubes in which 150 μl of homogenate was combined with 100 μl Krebs phosphate buffer containing the radio-

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ligand [^{125}I]cyanopindolol ([^{125}I]CYP; 10–200 pM saturation studies; 50–80 pM displacement studies), guanosine triphosphate (GTP) (0·1 mM), ascorbic acid (1 mM), EDTA (0·1 mM) and competing drug. Assays were terminated by rapid filtration after a 70 min incubation period at 37 °C. Specific binding was taken as the difference between total binding and that in the presence of propranolol (1 μM). Saturation and drug displacement data were analysed using two computer programs, EBDA (McPherson 1983a, b) which performed preliminary Scatchard, Hill and Hofstee analyses and created a file for LIGAND (Munson & Rodbard 1980), which was used to obtain final parameter estimates. The drugs used were (−)-isoprenaline bitartrate (Wyeth); procaterol hydrochloride (Warner Lambert); guanosine triphosphate (GTP), (−)-noradrenaline hydrochloride, (−)-adrenaline bitartrate (Sigma); (±)-*N*-*t*-butylnoradrenaline methansulfonate (Sterling Winthrop); practolol and ICI 118,551 (erythro-DL-1(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol) hydrochloride (Imperial Chemical Industries); desipramine hydrochloride and reserpine (Serpasil, Ciba-Geigy); L643,717-01J10 [(*S*)-2(P-[3-(3,4-dimethoxyphenethylamino)-2-hydroxypropoxy]-phenyl)-4-(2-thienyl)imidazole dihydrochloride] (Merck, Sharp & Dohme); phenoxybenzamine hydrochloride (Smith, Kline & French) and hydrocortisone sodium succinate (Glaxo). Stock solutions (10 mM) of drugs were prepared in either 10 mM HCl or distilled water. Phenoxybenzamine (0·1 M) was dissolved in acidified (1 μl 10 M HCl ml^{-1}) ethanol (95%). Dilutions were made using Krebs solution containing 1 mM ascorbic acid.

RESULTS

(−)-Isoprenaline elicited positive inotropic effects in guinea-pig and cat isolated left atrial preparations, mean pD_2 values of 8·4 ± 0·1 (n = 4) and 9·9 ± 0·1 (n = 4) respectively being obtained in the two tissues. While procaterol (1 nM–30 μM) was close to a full agonist (94 ± 5% (n = 4) of (−)-isoprenaline maximum response) and had a mean pD_2 value of 6·93 ± 0·14 (n = 4) in cat left atria, the drug produced only threshold positive inotropic responses in guinea-pig left atria (<5% of maximum response (−)-isoprenaline, n = 4) over the same concentration range. Fig. 1 shows mean concentration-effect curves for the two agonists in cat atrial preparations under control conditions and responses to procaterol after 40 min incubation with ICI 118,551 (0·1 μM). It is evident that ICI 118,551 produced a rightward shift of the lower portions of the curve to procaterol

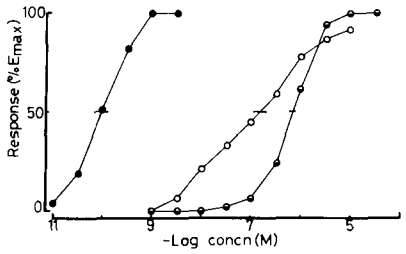

FIG. 1. Mean cumulative concentration-effect curves (n = 4) for the positive inotropic effects of (−)-isoprenaline (●) and procaterol in the absence (○) and presence (◒) of 0·1 μM ICI 118,551 in left atrial preparations from the cat. Each response is expressed as a percentage of the maximum response obtained with (−)-isoprenaline and bars indicate s.e.m. at the EC50 level.

(mean dose–ratio at EC25, 21 ± 6, n = 4) without affecting responses in the upper part of the curve. In other experiments (not shown) 0·1 μM ICI 118,551 produced a 2·9 ± 0·6 (n = 5)-fold parallel shift to the right in inotropic curves to (−)-isoprenaline in cat, but was without effect on (−)-isoprenaline responses in guinea-pig left atria (n = 4).

Concentration-effect curves for the inotropic effects of (−)-noradrenaline, (−)-adrenaline, (−)-isoprenaline and (±)-*N*-*t*-butylnoradrenaline were shifted to the right in a parallel manner by practolol in both guinea-pig and cat left atrial preparations. Table 1 shows mean pK_B values for practolol assessed from these rightward shifts. Whilst the pK_B values for practolol in guinea-pig left atria are independent ($P > 0.05$ Student's paired *t*-test) of the agonist used, those in cat left atria appear to be agonist-dependent. Thus, pK_B values with adrenaline and *N*-*t*-butylnoradrenaline, agonists displaying selectivity for β_2-adrenoceptor mediated responses in-vitro and in-vivo (Dowd et al 1977; Keh et al 1978), were significantly ($P < 0.05$ Student's paired *t*-test) lower than those for noradrenaline (β_1-adrenoceptor selective) and isoprenaline (non-selective).

In cat left atrial membrane preparations, [^{125}I]CYP (10–200 pM) bound in a saturable, reversible manner, without co-operativity to a single high affinity site. The mean dissociation constant (K_D) for [^{125}I]CYP was 22·5 ± 1·7 (n = 3) pM, the Hill coefficient 0·96 ± 0·07 (n = 3) and the maximal density of binding sites 102·3 ± 13·6 (n = 3) fmol mg^{-1} protein. The K_D value for [^{125}I]CYP in cat left atria is similar to that previously determined in guinea-pig left atrial membrane preparations (20·3 pM, McPherson et al 1984). The abilities of the selective β-adrenoceptor antagonists L643,717-01J10 (β_1-selective, Baldwin et al 1983) and ICI 118,551