the nine compounds were administered intravenously to anesthetized dogs to determine their effect on systemic arterial pressure. Compounds I, III, IV, V, and VII were administered in various dosage schedules ranging from 0.5 to 10.0 mg./Kg. Only compound I produced a marked change in blood pressure. Figure 2 shows the alteration in carotid arterial pressure produced by 1.6 mg./Kg. of compound I. It will be noted that there is an increase in both systolic and diastolic pressure, however, systolic pressure increased more than diastolic, thus increasing the pulse pressure too.

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A Potent α-Receptor Blocking Agent, SU-14542

By T. F. BURKS* and J. P. LONG

SU-14542 was studied in isolated cat and rabbit hearts and in *in situ* dog hearts for possible β-adrenergic receptor stimulating activity. It was determined that this compound has no demonstrable action on β receptors. Investigation of its effects on isolated mesenteric arteries demonstrated powerful α-adrenergic receptor blockade; SU-14542 was found to be 2–7 times more potent than phentolamine as an α-receptor blocking agent. The ID_{50} for SU-14542 in blocking epinephrine was determined to be $6.6 \times 10^{-9} M$.

IT HAS BEEN reported by Barrett *et al.* (1) that SU-14542, which is 3′-methoxy-4′ [(4-phenyl-1-piperazinyl)-butoxy]-acetophenone monohydrochloride, decreases arterial blood pressure in both anesthetized normotensive dogs and unanesthetized renal hypertensive dogs. Decreases in pressor responses produced by epinephrine, norepinephrine, and amphetamine were observed following oral administration of 0.20 and 1.80 mg./Kg. of SU-14542. These workers reported that the experimental compound does not possess ganglionic blocking activity but, since tachycardia was observed following administration of SU-14542, that it does possess β-adrenergic receptor stimulating activity.

Povalski *et al.* (2) reported that while 5.0 mg./Kg. SU-14542 given orally to anesthetized dogs produced a decrease in mean arterial blood pressure, cardiac output was not significantly altered. Rutledge *et al.* (3) found that SU-14542 increased femoral arterial blood flow in dogs but did not significantly increase renal blood flow.

In this communication evidence will be presented demonstrating that SU-14542 is a potent α-adrenergic receptor blocking agent that has no β-adrenergic receptor stimulating properties on dog, cat, or rabbit hearts.

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EXPERIMENTAL

Cat and Rabbit Hearts.—Dutch rabbits of either sex, weighing from 1.5–2 Kg. were sacrificed by cervical dislocation, and the beating hearts were removed and flushed through the aorta with a heparin–saline solution. An aortic cannula was tied into place and the hearts perfused in the usual Langendorf preparation (4) with Locke Ringer solution for isolated hearts warmed to 35–37° and aerated by bubbling 95% O_2–5% CO_2. Aortic pressure was maintained at 40–50 mm. Hg to ensure adequate coronary perfusion. Drugs were injected in 0.5–1 ml. vol. into the aortic cannula. Force of contraction was measured from a Grass Instrument Co. force - displacement transducer (FTO3C) and recorded on a Gilson (GME) polygraph. Heart rates were obtained by direct observation of recorder pen movement.

Cat hearts were prepared in a similar manner from cats of either sex weighing 1.5–3 Kg., anesthetized by intrathoracic administration of 30 mg./Kg. sodium pentobarbital.

Dog Hearts.—Mongrel dogs of either sex, weighing 10–12 Kg., were anesthesized by 15 mg./Kg. sodium thiopental and 250 mg./Kg. sodium barbital administered intravenously. Systemic blood pressure was measured from a carotid artery cannula by a Statham pressure transducer (P23AA) and recorded by an Offner type RS Dynograph. Drugs, in volumes of 0.1–1 ml., were injected through a cannula placed in a femoral vein. Both vagus nerves were sectioned in the cervical region and each animal was administered 20 mg./Kg. hexamethon-

TABLE I.—EFFECTS OF SU-14542 AND EPINEPHRINE ON HEARTS (MEANS ± S.E.)

Species	N[a]	Rate/min. Control	Epi[b]	P	Contractile Force, g. Control	SU[c]	P[d]	Control	Epi[b]	P	Control	SU[c]	P[d]
Rabbit	5	168 ± 14	202 ± 16	<0.01	164 ± 13	148 ± 11	<0.01	7 ± 0.9	14 ± 1.1	<0.01	7 ± 1.0	4 ± 1.0	<0.01
Cat	5	90 ± 8	138 ± 7	<0.01	94 ± 15	77 ± 12	<0.05	9 ± 1.6	24 ± 3.7	<0.01	8 ± 1.3	5 ± 1.5	<0.01
Dog	5	108 ± 7	144 ± 11	<0.01	106 ± 6	104 ± 5	N.S.[e]	123 ± 16	267 ± 47	<0.01	118 ± 18	111 ± 12	N.S.[e]

[a] N, number of animals used per experiment. [b] Epinephrine, 1 mcg./dose in cat and rabbit hearts; 1 mcg./Kg. in dogs. [c] SU-14542, 100 mcg./dose in cat and rabbit hearts; 1.0 mg./Kg. in dogs. [d] Analysis for decrease in response. [e] Not statistically significant.

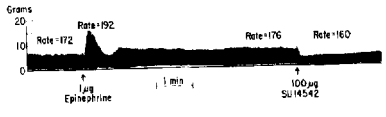

Fig. 1.—Effects of the standard β-receptor stimulant, epinephrine, and SU-14542 on rate and force of contraction of isolated rabbit heart. Note that epinephrine produced increases in both contractile force and rate. SU-14542 decreased both contractile force and rate.

ium bromide. The heart was exposed by a midline thoracic incision, the pericardium opened, and a Walton-Brodie strain gauge arch sewn directly on the myocardium. Contractile force was recorded by the Offner Dynograph. Heart rates were obtained by direct observation of recorder pen deflection.

Isolated Mensenteric Arteries.—Isolated arteries were prepared in the manner described by Rogers *et al.* (5). Dogs of either sex were anesthetized as above and the small intestine exposed *via* midline abdominal incision. Mesenteric arteries with branching fans of smaller resistance arteries and periarterial sympathetic nerves were removed from the animals and mounted in an organ bath where they were perfused with Krebs bicarbonate solution. The Krebs solution was aerated with 95% O_2–5% CO_2 and maintained at 37°. The arterial segments were immersed in a 150-ml. recirculating bath and perfused by use of a Sigmamotor model T-8 peristaltic infusion pump. Since flow was held constant, changes in perfusion pressure were directly proportional to changes in arterial resistance. Perfusion pressure was measured from a T tube between the pump and the artery by a Statham pressure transducer (P23AA) and recorded on an Offner Dynograph.

Stimulation of the periarterial sympathetic nerves was accomplished with a Grass Instrument Co. model S4 stimulator. Parameters of stimulation were within the following ranges: frequency 20–30 c.p.s., duration 5–20 msec., and at 6–15 v. for 1–10 sec. Epinephrine and norepinephrine dissolved in purified water were injected into the arterial cannula in volumes of 0.01–0.05 ml. and the blocking agents were injected directly into the bath fluid. Several test doses of the agonists were given and several stimulations performed until repeated challenge produced reproducible responses between 50–100 mm. Hg above the baseline pressure which was maintained at 80–120 mm. Hg.

After establishment of control responses, the antagonists were added to the bath and the agonists reapplied; this was repeated for a second dose of antagonists.

Chemicals.—Chemicals employed were *l*-epinephrine HCl, *l*-norepinephrine HCl (calculated as the base), phentolamine HCl, SU-14542, and hexamethionium bromide.

The isolated heart experiments were so designed that each heart served as its own control and the data were analyzed with the Student t test, paired comparisons (6). The data from the isolated arteries were analyzed by analysis of variance and a 2 × 2 parallel line bioassay (7). ID_{50} doses for the isolated arteries were calculated by the method of Litchfield and Wilcoxon (8). In all experiments a P value equal to or less than 0.05 was considered significant.

RESULTS

Hearts.—As can be seen from perusal of Table I, administration of epinephrine resulted in increases in both rate and force of contraction of the hearts in all species investigated. This is of course consistant with its well-known ability to stimulate β-adrenergic receptors. SU-14542 produced decreases in rate and/or force of contraction (Fig. 1). The effects of SU-14542 on the hearts were dose-related and doses ranging from 1–300 mcg. were applied to the cat and rabbit hearts; a 100-mcg. dose was chosen for the study. In the dogs, doses from 0.01–5.0 mg./Kg. were employed and 1.0 mg./Kg. was chosen for the study, this being a dose comparable to those used by other workers. In one instance a dog was prepared as described above except the vagi were left intact and hexamethonium was not administered. In this case, administration of 1.0 mg./Kg. SU-14542 resulted in increases in both heart rate and contractile force, the onset corresponding to the fall in arterial blood pressure. It was also interesting to note that when epinephrine was administered to the isolated hearts after SU-14542 there was no indication of β-receptor blockade when the response was compared to that produced by epinephrine before SU-14542.

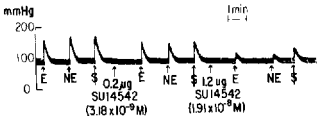

Fig. 2.—Demonstration of α-receptor blocking action of SU-14542. The stimuli applied were epinephrine (E), 0.1 mcg.; norepinephrine (NE), 0.2 mcg.; and sympathetic nerve stimulation (S with an arrow) 25 c.p.s., 5 msec./pulse, 10 v. for 3 sec. The mcg. doses and M concentrations of SU-14542 represent total bath concentration.

TABLE II.—EFFECTS OF SU-14542 AND PHENTOLAMINE ON ISOLATED MESENTERIC ARTERIES, INCREASE IN PERFUSION PRESSURE, mm. Hg

	N^a	Mean Response: Control	Low Dose[b]	High Dose[c]	R[d]
Epinephrine: phentolamine	8	74	56	14	...
SU-14542	8	59	45	8	2.27 (2.05–2.50)
Norepinephrine: phentolamine	8	74	54	10	...
SU-14542	8	63	30	6	2.40 (2.07–2.77)
Nerve stimulator: phentolamine	8	56	42	37	...
SU-14542	8	73	54	24	7.02 (6.34–7.84)

[a] N, number of animals per experiment. [b] Low dose of phentolamine was $8.41 \times 10^{-9}M$; SU-14542 was $3.18 \times 10^{-9}M$. [c] High doses (representing total concentration): phentolamine was $5.05 \times 10^{-8}M$; SU-14542 was $1.91 \times 10^{-8}M$. [d] Potency ratio (standard substance was phentolamine) with 95% fiducial limits as determined by parallel line bioassay.

TABLE III.—ANALYSIS OF VARIANCE–SU-14542 AND PHENTOLAMINE *Versus* EPINEPHRINE (RANDOMIZED COMPLETE BLOCK DESIGN)

Source of Variation	d.f.[a]	SS[b]	MS[c]	*F*[d]	*Pe*
Preparation	1	95	95	<1	NS[f]
Regression	1	12,207	12,207	89.10	<0.01
Parallelism	1	63	63	<1	NS[f]
Treatments (doses)	(3)	(12,364)	...	...	...
Animals (blocks)	7	2,711	387	2.82	<0.1 >0.05
Error	21	2,868	137	...	...
Total	31	17,943			

[a] Degrees of freedom. [b] Sum of squares. [c] Mean square. [d] F ratio. [e] Level of significance. [f] Not statistically significant.

Isolated Arteries.—SU-14542 was found to be a potent α-adrenergic receptor blocking agent, being some 2–7 times more potent than phentolamine (Table II). Preliminary experiments determined that $3.18 \times 10^{-9}M$ and $1.91 \times 10^{-8}M$ concentrations of SU-14542 were approximately comparable to $8.41 \times 10^{-9}M$ and $5.05 \times 10^{-8}M$ concentrations of phentolamine in blocking the responses to epinephrine and norepinephrine. It was also determined that the dose-response curve to SU-14542 was relatively flat, the optimal log interval between the high and low doses was found to be 0.8 (Fig. 2). In later experiments SU-14542 was determined to be some 7 times more potent than phentolamine in antagonizing responses to sympathetic nerve stimulation. As was expected, and as can be seen from Fig. 2 and Table II, both α-receptor blocking agents were more effective antagonists of exogenously administered catecholamines than in antagonizing sympathetic nerve stimulation.

Median inhibitory doses (ID_{50}) calculated for each of these α-receptor blocking agents against epinephrine with their 95% confidence limits were as follows: SU-14542, $6.6 \times 10^{-9}M$ ($2.3–19.1 \times 10^{-9}M$); phentolamine, $16.5 \times 10^{-9}M$ ($5.9–46.2 \times 10^{-9}M$).

Analysis of variance and parallel line bioassay provided evidence for good regression lines for each agent at the 0.8 log interval of doses, no deviation from parallelism and good matching of doses. A typical table of analysis of variance is provided in Table III.

DISCUSSION

From the data presented it may be concluded that SU-14542 is a potent antagonist of the α-adrenergic receptor stimulation resulting either from addition of exogenous catecholamines or from stimulation of sympathetic nerves in isolated mesenteric arteries. SU-14542 was determined to be some 2–7 times more effective than phentolamine as an α-receptor blocking agent, and, therefore, could well find use in experimental and perhaps in clinical situations where the use of such a property might be indicated. The relatively flat dose-response curve demonstrated for this agent may be beneficial to its employment in diverse circumstances.

It is evident, however, that this compound is not a stimulant of the β-receptors of cat, rabbit, or dog hearts. It is suggested that the heart stimulation observed by other investigators following administration of SU-14542 was mediated reflexly as compensation for the fall in arterial blood pressure resulting from the intense α-receptor blockade produced by this compound. The single experiment described above in which a dog with intact cardiovascular reflexes responded to SU-14542 by heart stimulation may be contrasted to the other animals with reflexes blocked by vagatomy and hexamethonium that failed to show any such heart stimulation.

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