Biochemical Pharmacology, Vol. 19, pp. 1221–1227. Pergamon Press. 1970. Printed in Great Britain

THE INFLUENCE OF PROPRANOLOL, INPEA,* IPROVERATRIL† AND SOME 1-NAPHTHYLETHYLAMINE DERIVATIVES ON THE MYOCARDIAL PHOSPHORYLASE ACTIVITY

JADWIGA ROBAK and RYSZARD GRYGLEWSKI

Department of Pharmacology, Medical Academy, Kraków, Poland

(*Received* 8 *July* 1969; *accepted* 15 *September* 1969)

Abstract—The antiarrhythmic or negative inotropic and chronotropic response to drugs may be the result of β-adrenergic blockade or the result of the quinidine-like action. The influence of the cardioactive drugs on the isoprenaline-induced phosphorylase activity in the cardiac muscle is decisive in this respect.

The influence of propranolol, INPEA, iproveratril and two 1-naphthylethylamine derivatives on the isoprenaline induced phosphorylase activity was studied. Rat hearts were perfused by the method of Langendorff during 20 min. The compounds were infused alone in the range of 0·5–500 μg doses and also 2 min after 0·1 μg isoprenaline (IP) was injected into the perfusion system. Samples of the muscle were taken 30 sec afterwards and the phosphorylase activity was determined. Control hearts were treated with IP alone. IP increased significantly the phosphorylase activity in cardiac muscle as compared with the control. Propanolol completely prevented the effect of IP on metabolism and reduced the mechanical effect of IP.

D(−)*N*-isopropyl-*p*-nitrophenyl-ethanolamine *d*(−)INPEA (5 μg) and *l*(+)INPEA (50 μg and 500 μg) were ineffective against the IP-induced stimulation of the heart. D(−)INPEA at 50 and 500 μg reduced the effect of IP on phosphorylase activity and blocked completely the pharmacodynamical action of IP on the heart muscle.

α-isopropyl-α-[(*N*-methyl-*N*-homoveratryl)-α-aminopropyl]-3,4-dimethoxyphenylacetonitrile (Iproveratril) at 0·5 and 2·5 μg depressed the cardiac activity but not the mechanical or metabolic effects of IP.

N-isopropyl-α-methyl-β-1-naphthylethylamine (S-870) (5 μg) and *N*-propyl-α-methyl-β-1-naphthylethylamine (S-931) (5 μg) did not influence the phosphorylase activity in the heart or the effects of IP.

THE β-adrenergic stimulation results in the activation of the adenylcyclase system and the increase in the intracellular concentration of 3′5′AMP.[28] This nucleotide activates ATP: phosphorylase phosphotransferase (EC 2.7.1.38) which transforms myocardial α-1,4-glucan:orthophosphate glucosyltransferase (EC 2.4.1.1)—so called phosphorylase—from the inactive form b into the active form a.[14]

Adrenomimetic drugs, e.g. isoprenaline (IP) increase the concentration of phosphorylase a in the perfused rat heart,[15] in the perfused guinea-pig heart[16–19] and *in vivo* experiments.[22] β-blocking agents, e.g. dichloroisoprenaline[12, 20] or pronethanol[3, 17, 18] prevent the activation of phosphorylase by IP.

* *N*-isopropyl-*p*-nitrophenyl-ethanolamine hydrochloride.
† α-isopropyl-α-[(*N*-methyl-*N*-homoveratryl)-α-aminopropyl]-3, 4-dimethoxyphenylacetonitrile.

Using the perfused rat heart, we investigated the influence of propranolol, INPEA, iproveratril and two 1-naphthylethylamine derivatives on the spontaneous and IP-induced activity of phosphorylase.

Propranolol and INPEA were used as the standard β-blocking agents.[1, 11, 13, 23–27, 31] We investigated the correlation between metabolic and contractile effects of the β-blockade on the cardiac muscle.

For closer inspection of this correlation three other drugs were used: iproveratril, which was claimed to be a β-blocking agent[8, 10] or to be deprived of this activity[6, 30] and the derivatives of 1-naphthylethylamine: S-870 and S-931 which were supposed to

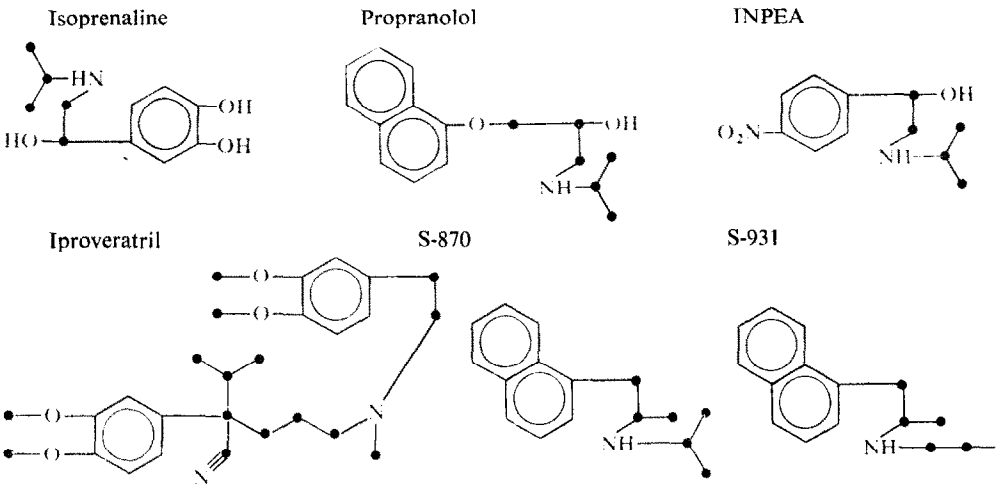

FIG. 1.

possess antiarrhythmic activity but no β-blocking properties.[9]

The similarity between the chemical structures of the investigated compounds as compared with IP is presented in Fig. 1.

MATERIALS AND METHODS

Compounds used

Glucose, Dextran, EDTA, Sodium fluoride, Ammonium molybdate were produced by Polish Reagent Factories.

Glycogen, from rabbit livers, was purified by the method of Sutherland and Wosilait.[32]

α-D-glucose-1-phosphate grade III disodium salt and Adenosine 5′-monophosphoric acid (5′-AMP) from yeast were kindly supplied by Sigma Chemical Company.

d-l.-isoprenaline sulphate (IP) (Novodrin) was kindly supplied by VEB Chemisches Werk Berlin-Grünau.

d,l(+) propranolol hydrochloride (PR) (Inderal) was kindly supplied by Imperial Chemical Industries, Macclesfield.

d(−) and *l*(+) *N*-isopropyl-*p*-nitrophenyl-ethanolamine hydrochloride (INPEA) was kindly supplied by Selvi et C., Milano, Italy.

α-isopropyl-α-[(*N*-methyl-*N*-homoveratryl)-α-aminopropyl]-3,4-dimethoxyphenyl-acetonitrile hydrochloride (IR) (Iproveratril, Isoptin, Verapamil) was kindly supplied by Knoll A.G. Ludvigshafen.

N-isopropyl-α-methyl-β-1-naphthyl-ethylamine (compound S-870) and *N*-propyl-α-methyl-β-1-naphthyl-ethylamine (compound S-931) were kindly supplied by Prof. K. Kelemen, University of Budapest. All doses were expressed as free bases.

Animals

144 male Wistar strain rats weighing 150–200 g were used in the experiments. The animals received food and water *ad lib.* The excision of the heart was done under light ethyl-ether anaesthesia.

Perfusion of the hearts

The method of Langendorff[21] modified by Wiegershausen[33] was used. The composition of the nutrient fluid was as follows: (%w/v) 0·86 NaCl, 0·03 KCl, 0·024 $CaCl_2$, 0·07 $NaHCO_3$, 0·2 glucose, 0·3 dextran. The fluid was oxygenated with pure oxygen and warmed at 37°. The perfusion was carried out under a hydrostatic pressure of 30 mm Hg.

The stabilisation of the phosphorylase activity was achieved during the first 20 min of perfusion. After the period of stabilisation, the drug solutions were injected into the perfusion system in a volume of 0·1 ml. The experimental group received the following drugs:

d,l(+) PR, S-870, S-931	5 μg
d(−) INPEA, *l*(+) INPEA	5 μg, 50 μg, 500 μg
IR	0·5 μg, 2·5 μg

30 sec after the drug infusions samples of the heart tissue were taken and the phosphorylase activity was determined. The enzyme activity in this group was compared with untreated controls.

The main experimental group consisted, however, of the hearts, which 120 sec after the drug treatment, were injected with 0·1 μg IP. Thirty sec later, samples of the heart muscle were taken and the phosphorylase activity was determined. The enzyme activity was compared with the activity of the controls treated with IP alone.

The contractile force of the perfused heart was measured mechanically on the smoked drum using semiisometric lever (1 g weight = 13 mm deviation). The influence of the tested substances on the cardiac contractile force was expressed using the following formula:

$$\frac{a-b}{b} \cdot 100$$

where a is the amplitude after treatment, and b is the amplitude before treatment.

Determination of phosphorylase

The method was based on that described by Kukovetz.[19] Samples of the heart muscle (about 100 mg) were cut from the ventricles, and dashed into a mixture of solid carbon dioxide and ethanol. The frozen samples were blotted, weighed and pulverized in a precooled mortar containing 3 ml of the frozen solution (−10°) of 0·02 M sodium fluoride and 0·001 M EDTA for 100 mg of tissue. The melted mixture was then diluted 5-fold with the above mentioned NaF-EDTA solution. It contained 6·6 mg of the tissue per ml. The homogenate was used without centrifugation as the source of enzyme.

Two 0·5 ml samples of the enzyme preparation were incubated with 0·5 ml of substrates solution at 30°. The substrates solution contained: 2% w/v of glycogen and

0·97% w/v of α-D-glucose-1-phosphate for the estimation of phosphorylase a, and the same with addition of 0·066% w/v 5'AMP for estimation of total phosphorylase.

The reaction lasted 5 min and was terminated by adding 5 ml of 1 N H_2SO_4. Inorganic phosphate, formed by acting of phosphorylase on substrate, was determined by the method of Fiske and SubbaRow[5, 29] using a Pulfrich colorimeter supported with Elfo-equipment. Enzyme activity was expressed in units as described by Cori *et al.*[2] The enzyme activity in samples deprived of 5'AMP was expressed as percentage of the enzyme activity in samples containing 5'AMP. Since none of the tested substances did significantly influence the level of total phosphorylase, the results were expressed only in percentage of phosphorylase a. Therefore the terms "phosphorylase activity" and "percentage of phosphorylase a" were used as interchangeable logical synonyms.

All data were analysed by the Student's *t*-test.

RESULTS

IP at the dose of 0·1 μg increased significantly the phosphorylase activity and the contractile force of the heart (Table 1).

TABLE 1. THE INFLUENCE OF ISOPRENALINE (IP), PROPRANOLOL (PR) AND *d*(−)*N*-ISOPROPYL-*p*-NITROPHENYL-ETHANOLAMINE (INPEA) ON THE PHOSPHORYLASE ACTIVITY AND CONTRACTILE FORCE OF THE PERFUSED RAT HEART

Treatment		Number of determinations		Percent of phosphorylase a ±S.E.	Percent of increase in contractile force ± S.E.
Drug	Dose (μg)	Phosphorylase activity	Contractile force		
Perfusion fluid	—	36	20	22·0 ± 1·3	43 ± 5
IP	0·1	10	16	68·8 ± 6·5*	199 ± 54†
PR	5·0	9	11	22·9 ± 6·2	41 ± 12
d(—)INPEA	5·0	6	12	21·0 ± 3·7	40 ± 9
d(−)INPEA	50·0	6	12	25·1 ± 3·3	62 ± 18
IR°	0·5	—	6	—	40 ± 14
IR	2·5	—	9	—	−73 ± 8

Drugs were given after 20 min of perfusion. Thirty sec later the determinations were done. The statistical significance against the control was denoted: * = $P < 0{\cdot}001$ † = $0{\cdot}01 > P > 0{\cdot}001$. ±S.E. means standard error.

PR alone at the dose of 5 μg did not affect the mechanical and metabolic activities of the heart (Table 1). However, pretreatment with the same dose of PR abolished the metabolic response to IP and resulted in a marked diminution of the mechanical effect of IP (Table 2).

D(−) INPEA alone at 5 and 50 μg did influence neither percentage of phosphorylase a or contractile force of the heart (Table 1).

D(−) INPEA was investigated at three doses against IP stimulation: 5, 50 and 500 μg. An increasing inhibition of the metabolic and mechanical response of the heart muscle to IP-stimulation was observed, but the mechanical response was blocked more dramatically than the metabolic response as the doses of *d*(−) INPEA increased (Table 2).

L(+) INPEA was investigated at 50 and 500 μg. It blocked neither mechanical nor metabolic effects of IP (Table 2). The differences between the IP-blocking activities of the two isomers of INPEA at 50 and 500 μg were highly significant.

IR at 0·5 and 2·5 μg did not diminish the effects of IP on the heart muscle. The contractile force of the heart was even increased when IP was applied after the higher dose of IR (Table 2). IR in the dose of 2·5 μg resulted in a marked depression of the mechanical activity of the heart (Table 1).

Compounds S-870 and S-931 at the dose of 5 μg did not change significantly the influence of IP on the metabolism and heart contractibility (Table 2).

TABLE 2. THE INFLUENCE OF PROPRANOLOL (PR), *d*(−) AND *l*(+) *N*-ISOPROPYL-*p*-NITROPHENYL-ETHANOLAMINE (INPEA), α-ISOPROPYL-α[(*N*-METHYL-*N*-HOMOVERATRYL)-α-AMINO-PROPYL] 3,4-DIMETHOXYPHENYL-ACETONITRILE (IR), *N*-ISOPROPYL-α-METHYL-β-1-NAPHTHYLETHYLAMINE (S-870) AND *N*-PROPYL-α-METHYL-β-1-NAPHTHYLETHYLAMINE (S-931) ON THE ACTION OF ISOPRENALINE ON THE PHOSPHORYLASE ACTIVITY AND ON THE CONTRACTILE FORCE OF THE PERFUSED RAT HEART

Treatment		Number of determinations		Percent of phosphorylase a ±S.E.	Percent of increase in contractile force ± S.E.
Drug	Dose (μg)	Phosphorylase activity	Contractile force		
Perfusion fluid	—	10	16	68·8 ± 6·5	199 ± 54
PR	5	10	8	22·3 ± 4·7*	92 ± 29
d(−)INPEA	5	6	5	50·0 ± 5·5	227 ± 88
d(−)INPEA	50	10	10	37·0 ± 6·0†	81 ± 35
d(−)INPEA	500	6	6	32·5 ± 2·9*	19 ± 12†
l(+)INPEA	50	8	8	61·0 ± 6·7	202 ± 67
l(+)INPEA	500	6	6	51·0 ± 7·6	257 ± 44
IR	0·5	6	6	54·0 ± 8·8	330 ± 100
IR	2·5	6	9	54·0 ± 2·8	1771 ± 942
S-870	5	10	7	60·0 ± 7·8	121 ± 59
S-931	5	13	9	54·2 ± 6·3	195 ± 46

Drugs were infused after 20 min of perfusion. Two minutes later 0·1 μg isoprenaline was injected and after 30 sec the determinations were done. The statistical significance against the control treated with 0·1 μg isoprenaline alone was denoted: * = $P < 0{\cdot}001$, † = $0{\cdot}01 > P > 0{\cdot}001$.
±S.E. means standard error.

DISCUSSION

It is known that IP increases the content of phosphorylase a in the perfused cardiac muscle and augments its contractile force.[15–17]

We found that PR at the dose of 5 μg abolishes the IP-induced activation of phosphorylase in the perfused heart of rat. At the same time the mechanical effect of IP is not completely blocked. Similar to our metabolic results were obtained *in vivo*: PR at the dose 2 mg/kg blocked the activation of the rat heart phosphorylase by noradrenaline.[25, 26]

INPEA, another β-blocking agent is claimed to be deprived of non-specific PR-like properties[4] but to possess a trace of an intrinsic adrenergic activity.[23, 24] If this last opinion is true, INPEA should increase the activity of phosphorylase as all adrenergic stimulants do.[15, 22] In our experiments, *d*(−) INPEA, at the dose of 50 μg, does not influence the content of phosphorylase a in the cardiac muscle, but stimulates slightly the contractibility of the heart.

The β-blocking activity of INPEA is confined to its *d*(−) isomer.[1, 24, 27] In our experiments, INPEA, at the same dose (5 μg) as *d,l*(±) PR, does not block the metabolic or mechanical effects of IP. D(−) INPEA, at the dose of 50 μg, partially reduces

the metabolic and mechanical responses to IP, and at the dose of 500 μg, it blocks completely the mechanical response to IP whereas the metabolic response to IP is only partially reduced.

It seems that *d*(−) INPEA antagonizes more readily the mechanical than the metabolic response to IP in the perfused rat heart.

Murmann[23] was not able to find antagonism between IP and *d*(−) INPEA in anaesthetized rats. In our experiments on the perfused rat hearts, the β-blocking activity of *d*(−) INPEA against IP is about 100 times lower than the β-blocking activity of *d,l*(±) PR.

L(+) INPEA up to the dose of 500 μg does not antagonize the metabolic and mechanical effects of IP on the isolated rat heart, thus confirming the stereospecificity of the β-blocking activity of INPEA.[1, 24, 27]

The action of IR on β-adrenergic receptors is controversial. According to the definition of Fitzgerald and Barret[6] a β-blocker is a substance which antagonizes the positive chronotropic and inotropic effect of exogenous adrenaline or IP on the cardiac muscle but does not prevent the positive inotropic effects of Digitalis, calcium ions and aminophylline. IR antagonizes the cardiac effects of all these stimulants[6] and thus Fitzgerald and Barret did not classify IR as β-blocking agent. The results presented by Schaumann *et al.*[30] and Fleckenstein *et al.*[7] are in favour of this opinion. However, Heim and Walter[10] found that IR prevented the metabolic effects of anoxia and Haas[8] reported that IR (2·5 μg) reduced the inotropic positive response to IP (0·01 μg) in the perfused guinea-pig heart. Our results lead to the conclusion that IR has no β-blocking activity. IR at the dose of 2·5 μg exerts a strong negative inotropic effect on the perfused rat heart, but it does not prevent IP-induced activation of phosphorylase. IR paradoxically intensified the mechanical response to IP.

1-naphthylethylamine derivatives: S-870 and S-931 possess antiarrhythmic and local anaesthetic activities as potent as PR but do not block the IP-induced cardiac stimulation. Unlike PR they do not depress the activity of the cardiac muscle.[9] We confirmed that S-870 and S-931 do not change the mechanical response of the cardiac muscle to IP, and also we stated that the metabolic response of the cardiac muscle to IP is not diminished by S-870 and S-931.

Several pharmacodynamical[1, 7–9, 11, 13, 16–18, 22–24, 30] and metabolic[3, 7, 10, 12, 16–18, 20, 22–25, 30] methods are used in the investigation of the β-blocking activity, but only the experiment on the perfused animal heart allows to determine and to compare at the same moment the metabolic and the mechanical responses to β-stimulation or β-blockade. Our experiments confirmed that the simultaneous estimation of the phosphorylase activity and the contractile force of the heart may be a valuable test for β-blockers. Some investigated substances [*d,l*(±) PR, *d*(−) INPEA] block both metabolic and pharmacodynamical responses to β-stimulants. In the case of IR, S-870, S-931 and *l*(+) INPEA there are no pharmacodynamical or metabolic signs of β-blockade.

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