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INFLUENCE OF INPEA, PINDOLOL AND PROPRANOLOL ON THE CHRONOTROPIC AND METABOLIC RESPONSES TO β-ADRENERGIC STIMULATION IN INTACT RATS

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Abstract—A study was made of the effect of isoprenaline given intraperitoneally to rats on the chronotropic effect and the activation of myocardial phosphorylase. Pretreatment with three β-blocking agents: pindolol, propranolol and INPEA was carried out to separate metabolic and chronotropic effects of isoprenaline on heart.

Isoprenaline at 0·01–40 mg/kg augmented heart rate, but the activation of phosphorylase started only from the dose of 0·02 mg/kg. Metabolic effect of isoprenaline reached the maximum at a lower dose (0·1 mg/kg) than chronotropic effect of isoprenaline did (1 mg/kg).

Pindolol at 0·03 mg/kg, propranolol in doses 0·5–5 mg/kg and INPEA at a dose of 50 mg/kg slowed heart rate but did not influence phosphorylase activity. INPEA in doses 0·5–5 mg/kg, accelerated heart rate, but did not activate myocardial phosphorylase.

β-Blocking potency of all three drugs was nearly the same with respect to metabolic and chronotropic response to isoprenaline, but only when measured at the level of 50 per cent of inhibition. ID_{50} were: pindolol—0·006 mg/kg, propranolol—0·33 mg/kg and INPEA—2·8 mg/kg, however distinct differences between metabolic and chronotropic blockade in other doses of propranolol and INPEA were found.

Linear dose dependent β-adrenergic blockade was induced with respect to metabolic and chronotropic effects by pindolol, with respect to chronotropic effect by propranolol and in respect to metabolic effect by INPEA. The blockade of metabolic effect by propranolol and the blockade of chronotropic effect by INPEA occurred abruptly from the zero level to maximal level when the dosage of these drugs was doubled.

THE INTERRELATIONSHIP between mechanical and metabolic effects of cardiotropic drugs is extensively investigated.[1–11] Inotropic or chronotropic effects and activity of myocardial α-1,4-glucan: orthophosphate glucosylotransferase (EC 2.4.1.1)—so called phosphorylase is usually correlated. Percentage of phosphorylase a is considered to be an indicator of the content of cyclic 3′5′AMP in myocardium.[12] It is believed that the change in contractility or in pacing occurs simultaneously with the acceleration of cardiac metabolism, because these effects result from the increase of formation of 3′5′AMP.[12–16] However, some authors[1–4,6,8–11,17] claimed to be able to separate the metabolic effect from the mechanical effect of β-adrenergic stimulation. We compared the positive chronotropic effect of isoprenaline in rats with its ability to activate myocardial phosphorylase. The pretreatment of rats with a wide range of doses of three β-blocking agents propranolol (PR),[18–22] INPEA[20,22–26] and pindolol (LB-46)[19,27–29] was carried out in an attempt to separate chronotropic and metabolic effects of isoprenaline.

MATERIALS AND METHODS

Compounds used

Sodium fluoride, ammonium molybdate, sodium pyrosulphite, EDTA, trichloracetic acid were produced by Polish Reagent Factories. Sodium amobarbital (Sodium Amytal) was produced by Lilly Company Ltd. England.

Glycogen from rabbit livers was purified by the method of Sutherland Wosilait.[30]

α-Glucose-1-phosphate di-Na salt pure was obtained from Koch and Light Laboratories Ltd. England.

The following reagents and drugs were kindly offered by the appropriate firms: Adenosine 5′-monophosphoric acid (5′AMP) from yeast—Sigma Chemical Company. DL (±)-isoprenaline sulphate (IP, Novodrin)—VEB Chemisches Werk Berlin Grünau. DL (±)-propranolol hydrochloride (PR, Inderal)—Imperial Chemical Industries Macclesfield. D (—)-*N*-isopropyl-*p*-nitrophenyl-ethanolamine hydrochloride (INPEA) —Selvi et C. Milano Italy. DL (±)4-[2-hydroxy-3-isopropylamino-propoxy]-indole (LB-46, pindolol)—Sandoz Basel.

All doses were expressed as free bases.

Animals

Male Wistar strain rats weighing 200–300 g were used in the experiments. The animals received food and water *ad lib.*

Determination of the chronotropic effect

The rats were anaesthetized with sodium amobarbital (100 mg/kg) intraperitoneally. Five min later the animals were injected with β-blocking agents in a volume of 5 ml/kg or with the same volume of saline. Fifteen min later ECG was recorded and immediately IP (0·1 mg/kg) was injected intraperitoneally. Fifty-five sec later ECG was recorded again. The heart rate was calculated from the ECG recording tape. This moved with the speed 100 mm/sec and 500 mm pieces were used for a calculation.

Determination of phosphorylase activity

The rats were narcotized with amobarbital and injected with IP, β-blockers or both as described above. The chest was opened and the apical part of the heart was removed for further procedure. The method of determination of phosphorylase activity was essentially the same as described by Diamond and Brody.[31] The only difference was the use of pentane instead of isopentane to freeze the heart slices and the change of the incubation period from 30 to 14 min. Inorganic phosphate was determined by the method of Fiske and Subba Row.[32] Enzyme activity was expressed in μmoles of inorganic phosphate which was formed during the incubation period and calculated for 1 g of tissue and for 1 min. The percentage of phosphorylase a was calculated from the enzyme activity in samples deprived of 5′AMP and in samples containing 5′AMP. Since none of the tested substances significantly influenced the level of total phosphorylase the results were expressed in percentage of phosphorylase a. All data were analysed by the Student's *t*-test. ID_{50} values for β-blocking drugs were determined graphically.

TABLE 1. THE INFLUENCE OF ISOPRENALINE ON THE HEART RATE AND PHOSPHORYLASE ACTIVITY IN RATS

	Dose of isoprenaline (mg/kg)						
	0	0·005	0·01	0·02	0·1	1·0	40·0
Heart rate in beats per min ± S.E.	316 ± 4 $n = 113$	331 ± 7 $n = 9$	343 ± 11† $n = 18$	367 ± 9* $n = 12$	402 ± 8* $n = 24$	452 ± 8* $n = 26$	468 ± 8* $n = 6$
Phosphorylase activity % a ± S.E.	15·5 ± 2·7 $n = 19$	— —	13·8 ± 5·2 $n = 6$	41·8 ± 2·8* $n = 6$	59·4 ± 5·0* $n = 7$	66·3 ± 3·9* $n = 7$	68·2 ± 5·5* $n = 6$

Rats were narcotized with amobarbital (100 mg/kg) and injected intraperitoneally with 5 ml/kg of saline. Fifteen min later ECG was recorded and isoprenaline was injected intraperitoneally. Fifty-five sec later ECG was registered again and then the chest was opened and a sample of the heart muscle was removed to estimate phosphorylase activity. S.E. stands for standard error and n for number of rats in a group. The statistical significance against the control group was denoted:

* $P < 0{\cdot}001$.

† $0{\cdot}05 > P > 0{\cdot}01$.

RESULTS

In the rats under investigation the total cardiac phosphorylase was 38 ± 0·7 μM P/g/min (n = 123), per cent of phosphorylase a was 15·5 ± 2·7 (n = 19) and heart rate was 316 ± 4 beats/min (n = 113).

The influence of different doses of IP on the phosphorylase activity and on the heart rate is shown in Table 1 and in Fig. 1. IP in a dose of 0·01 mg/kg did not change the activity of phosphorylase, but slightly accelerated heart rate. A 10-fold higher dose of IP resulted in nearly the maximal activation of phosphorylase whereas still 10-fold higher dose of IP was necessary to cause the maximal chronotropic effect.

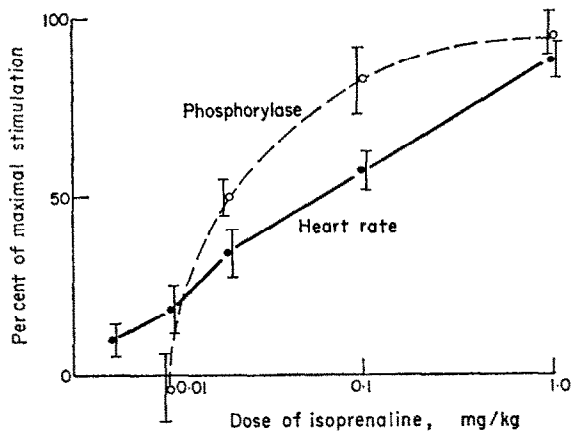

FIG. 1. Per cent of maximal stimulation of heart rate (HR) ●—● and phosphorylase activity (CP) ○—○ by isoprenaline (IP) in rats.
Each point was found according to the equation: $(m - c/M_x - c) \times 100$, where P is per cent of maximal stimulation, M_x is a value of HR or CP obtained after injection of IP in a supramaximal dose 40 mg/kg, m is value of HR or CP obtained after injection of an appropriate dose of IP and c is control value of HR or CP. Bars represent values of 2SE for each point. Number of estimations and actual value of HR and CP are presented in Table 1.

The influence of β-blocking agents LB-46, PR and INPEA on phosphorylase activity and on the heart rate is presented in Table 2. In a wide range of doses none of the tested drugs changed (at the level $P < 0{\cdot}01$) the per cent of phosphorylase a significantly as compared with the control.

The influence of β-blockers on the heart rate was not uniform. LB-46 did not change heart rate except of the bradycardia observed in the highest dose (0·03 mg/kg) of LB-46. PR in doses higher than 0·2 mg/kg induced dose dependent negative chronotropic effect. INPEA exerted a distinct positive chronotropic effect starting from the lowest dose of 0·5 mg/kg. This cardiostimulant effect of INPEA was not dose dependent being rather the same in the range of doses between 0·5 and 5 mg/kg. INPEA in the highest dose of 50 mg/kg had no cardiostimulant effect but it was slightly cardiodepressive.

The protective action of β-blockers on IP-induced cardiac stimulation is shown in Table 3 and in Fig. 2.

TABLE 2. THE INFLUENCE OF β-BLOCKING AGENTS ON THE HEART RATE AND PHOSPHORYLASE ACTIVITY IN RATS

Drug	Dose (mg/kg)	Number of determinations: Heart rate	Number of determinations: Phosphorylase activity	Heart rate (beats/min ± S.E.)	Phosphorylase activity (% a ± S.E.)
Saline	5 ml/kg	113	19	316 ± 4	15·5 ± 2·7
Pindolol	0·0003	15	8	341 ± 13	6·0 ± 2·5‡
	0·001	13	6	317 ± 15	12·5 ± 2·5
	0·003	18	6	323 ± 12	14·0 ± 6·7
	0·01	12	6	337 ± 11	10·8 ± 3·9
	0·03	5	6	252 ± 10*	11·3 ± 2·7
Propranolol	0·01	13	—	324 ± 10	—
	0·1	15	8	323 ± 10	8·2 ± 2·0‡
	0·2	16	6	304 ± 8	10·2 ± 4·1
	0·5	26	10	273 ± 5*	8·1 ± 2·7
	5·0	5	10	221 ± 17*	13·1 ± 3·7
INPEA	0·5	14	7	357 ± 14†	7·4 ± 4·3
	2·0	14	12	377 ± 10*	18·1 ± 3·6
	5·0	13	6	372 ± 11*	10·2 ± 4·5
	50·0	6	6	296 ± 19	16·0 ± 2·9

Rats were narcotized with amobarbital (100 mg/kg) and injected intraperitoneally with saline or with β-blockers. Fifteen min later ECG was recorded and then the chest was opened and a sample of cardiac muscle was removed to estimate phosphorylase activity. S.E. stands for standard error. The statistical significance against the control was denoted:

* $P < 0{\cdot}001$.
† $0{\cdot}01 > P > 0{\cdot}001$.
‡ $0{\cdot}05 > P > 0{\cdot}01$.

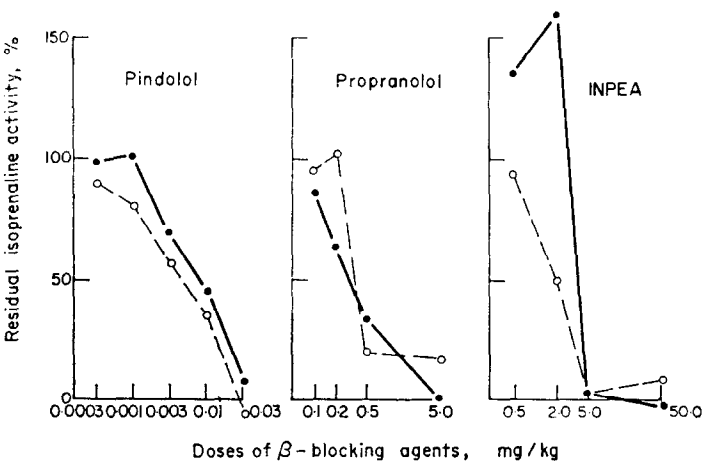

FIG. 2. The influence of pindolol, propranolol and D (—)-isopropyl-*p*-nitrophenyl-ethanolamine hydrochloride (INPEA) on the isoprenaline induced heart rate (HR) ●—● and myocardial phosphorylase activity (CP) ○—○ in rats.

Ordinate: Residual percentage of isoprenaline activity. It was calculated using following formula: $100 - \frac{IP - (B+IP)}{IP - B} \times 100$, where IP are the values of HR and CP after injection of 0·1 mg/kg of isoprenaline, (B + IP) are the values of HR and CP after β-blocking agent and isoprenaline, and B are the values of HR and CP after an injection of β-blocking agent alone. Number of estimations and actual values of HR and CP are presented in Tables 2 and 3.

Abscissa: Doses of β-blocking agents in log scale.

TABLE 3. THE INFLUENCE OF β-BLOCKING AGENTS ON THE ISOPRENALINE-INDUCED INCREASE IN THE HEART RATE AND PHOSPHORYLASE ACTIVITY

Drug	Dose (mg/kg)	Number of determinations: Heart rate	Number of determinations: Phosphorylase activity	Heart rate (beats/min ± S.E.)	Phosphorylase activity (% a ± S.E.)
Saline + 0·1 mg/kg isoprenaline	—	24	7	402 ± 8*	59·4 ± 5·0*
Pindolol	0·0003	15	6	401 ± 18‡	54·3 ± 1·9*
+ 0·1 mg/kg	0·001	13	6	404 ± 10*	50·0 ± 2·0*
isoprenaline	0·003	18	6	378 ± 9*	38·7 ± 0·9*
	0·01	12	7	367 ± 11	28·3 ± 6·5‡
	0·03	5	6	266 ± 8	8·8 ± 2·3
Propranolol	0·01	13	—	396 ± 9*	—
+ 0·1 mg/kg	0·1	15	7	391 ± 9*	56·3 ± 2·6*
isoprenaline	0·2	12	6	366 ± 5*	60·1 ± 8·6*
	0·5	24	6	317 ± 8*	21·1 ± 4·9
	5·0	5	6	221 ± 17	20·3 ± 5·9
INPEA	0·5	14	7	418 ± 13*	56·0 ± 2·6*
+ 0·1 mg/kg	2·0	14	8	417 ± 9†	35·0 ± 4·5*
isoprenaline	5·0	12	7	373 ± 11	12·9 ± 5·8
	50·0	6	6	294 ± 18	15·7 ± 4·6

Rats were narcotized with amobarbital (100 mg/kg) and injected intraperitoneally with saline or β-blockers. Fifteen min later isoprenaline (0·1 mg/kg) was injected intraperitoneally. In the relapse of 55 sec ECG was registered, the chest was opened and a sample of heart muscle was removed to estimate phosphorylase activity.

S.E. stands for standard error.

The statistical significance against the same dose of β-blocker alone (Table 2) was denoted:

* $P < 0{\cdot}001$.

† $0{\cdot}01 > P > 0{\cdot}001$.

‡ $0{\cdot}05 > P > 0{\cdot}01$.

For phosphorylase activity the effect of β-blocker alone was assumed to be average of all doses of all β-blockers = 11·4 ± 1·0% ($n = 97$).

LB-46 was the strongest β-blocker among tested drugs (ID_{50} was between 0·005 and 0·008 mg/kg). Chronotropic action of IP was a little bit less effectively blocked by LB-46 than metabolic activity of IP, but both cardiac effects of IP were blocked by LB-46 in dose-dependent, parallel and linear manner.

PR had ID_{50} = 0·3–0·4 mg/kg. Chronotropic effect of IP was blocked by PR in the dose range of 0·1–5 mg/kg in a dose dependent linear way. Dose–response curve for blockade of chronotropic effect of IP by PR was parallel to the analogous curve obtained for LB-46. Unlike in case of LB-46 the blockade of metablic effect of IP by PR was not linearly dose dependent. PR in the dose 0·1 and 0·2 mg/kg had no effect on IP-induced phosphorylase activation, whereas PR in dose of 0·5 and 5 mg/kg exerted its maximal blocking effect. However, this maximal metabolic blockade by PR was not the complete blockade, it reached only 80 per cent of full possibilities.

INPEA had ID_{50} = 2–4 mg/kg. INPEA in doses 0·5 and 2 mg/kg potentiated the positive chronotropic effect of IP by about 50 per cent. INPEA in doses 5 and 50

mg/kg completely blocked chronotropic effect of IP. A linear, dose-dependent blockade of IP-induced phosphorylase activation by INPEA (dose range 0·5–5 mg/kg) was observed.

DISCUSSION

In our experiments the content of phosphorylase a in control hearts was about 15 per cent. This value is considerably higher than those (0·5–4 per cent) cited by McNeill and Brody,[33–37] whereas the total phosphorylase content was the same. It was probably because of our inability to remove a sample of heart in such a short time as McNeill and Brody did (less than 3 sec). However, a number of authors[5,6,13,38] quoted the similar percentage of phosphorylase a to ours.

It is well known that IP activates phosphorylase in isolated perfused heart[14,16,38–40] or in hearts of open-chest animals.[41]

This effect of IP has been little investigated in intact animals.[35] We have decided therefore to investigate the influence of IP on cardiac phosphorylase activity and on the heart rate of intact rats.

Blinks and Kaumann[18,20] and Bristow and Green[42] found that the dose–response curves for inotropic and chronotropic effects of IP are similar. Although this point of view is not accepted uniformly[3,8] we assumed that estimation of chronotropic or inotropic response to IP may be used as an interchangeable indicative measure of an increased mechanical performance of the heart due to β-adrenergic stimulation.

It was found that small doses of IP (10 μg/kg) increased heart rate but did not influence phosphorylase activity. Mayer *et al.*[17] found in dogs that adrenaline in a dose of 0·1 μg/kg augmented cardiac contractile force without activation of phosphorylase. This observation was confirmed in isolated rat heart treated with adrenaline in doses 25–50 ng[13,43] and in isolated guinea pig hearts treated with noradrenaline in doses 12–65 ng[8] but not with isoprenaline in doses of 0·5–5 ng.[8] Kukovetz and Pöch[8] explained this phenomenon assuming that noradrenaline activates adenylcyclase system more slowly than IP does, therefore a minute amount of 3′5′AMP (formation of which is initiated by noradrenaline) are destroyed by phosphodiesterase (EC 3.1.4.1) before 3′5′AMP itself would be able to activate phosphorylase b-kinase (EC 2.7.1.38). The accumulation of 3′5′AMP in the heart under the influence of IP goes much faster and so both mechanical and metabolic effects of IP are always parallel. Results presented in this paper are not in favour of the hypothesis of Kukovetz and Pöch because low doses of IP are able to separate mechanical and metabolic effects of IP the same as low doses of noradrenaline do. According to Hammermeister *et al.*[44] the above phenomenon is common to all catecholamines and may be explained by the fact that myocardial fibers are more readily triggered by 3′5′AMP than phosphorylase b-kinase is.

In our experiments the increase in heart rate was linearly dependent on the dose of injected IP, whereas phosphorylase activity rose rather hyperbolically, e.g. IP in a dose 0·1 mg/kg stimulated heart rate in about 55 per cent of maximal response, while phosphorylase activity rose up to 85 per cent of the maximal response. These results obtained *in vivo* are discrepant with the results of Kukovetz and Pöch[8] obtained in perfused rat hearts. It may be interpreted that mechanical and metabolic effects of β-stimulation *in vivo* are not parallel or alternatively that in intact animals numerous

neuronal and humoral reflexes limit the possibility of an excessive acceleration of the heart rate.

All three investigated β-blocking agents did not decrease phosphorylase activity. The same phenomenon was described for PR.[9,35,37]

Heart rate was slowed by PR even in the lowest β-blocking dose, while INPEA and LB-46 produced negative chronotropic effect only in the highest dose used. Negative inotropic effect was described for high doses of LB-46,[45] PR[18,20] and INPEA.[18,20] It may be assumed that negative chronotropic effect of β-blocking agents depends mainly on their membrane activity[29,45] because it has been shown that β-adrenolytic drugs similarly to quinidine[46] and chloropromazine[21] depress mechanical performance of the heart but do not influence phosphorylase activity.

Sympathomimetic action of LB-46 is controversial, e.g. it was found that LB-46 possessed sympathomimetic activity in isolated atria,[27,47] in catecholamine depleted rat hearts[48] and in isolated tracheal chain,[28] but not in intact animals[19,27] nor in isolated papillary muscle.[49] We were not able to find any sympathomimetic activity of LB-46 in intact rats.

INPEA in a wide range of doses strongly accelerated heart rate. Kaumann and Blinks,[20] and Grodzińska[50] described a positive inotropic effect of INPEA, Somani,[26] Murmann *et al.*[24,25] and Barrett and Carter[48] described the positive chronotropic effect of this compound. We found that increase in cardiac work by INPEA was not accompanied by the catecholamine-like increase in cardiac phosphorylase activity in intact rats or in perfused rat hearts.[9] In our opinion administration of INPEA results in an increase in contractility of myocardium and an increase in conductivity of pacemaker in an unspecific way, but not due to its intrinsic sympathomimetic activity. If the "sympathomimetic" properties of INPEA would be mediated by adrenoreceptors the phosphorylase activity should be elevated in a parallel manner to activation of mechanical performance of the heart by INPEA. But it is not the case. The supposition that the metabolic effect of INPEA is too short to be observed should be rejected because Williamson and Jamienson[11] and Cheung and Williamson[2] have shown that mechanical effect of β-adrenergic stimulation lasts shorter than the elevation of cardiac phosphorylase a. It was claimed by Janiec and Chruściel[51] that INPEA may inhibit uptake of noradrenaline by heart slices, while some other β-blocking agents were supposed to release catecholamines from the cardiac tissue.[28,47] This indirect mechanism of action of INPEA on hearts of intact rats may be excluded because both cocaine[34] and tyramine[15,36,52] activate cardiac phosphorylase.

The specific pharmacological activity of β-blocking agents measured at the level of 50 per cent blockade (ID_{50}) of chronotropic and metabolic responses to intraperitonal injection of IP in a dose of 0·1 mg/kg was found as follows:

β-blocking agent	ID_{50} chronotropic response	ID_{50} metabolic response
LB-46	0·0079 mg/kg	0·0047 mg/kg
PR	0·3 mg/kg	0·37 mg/kg
INPEA	3·64 mg/kg	2·0 mg/kg

It may be seen that there are no essential differences between ID_{50} values for chronotropic and metabolic blockade of β-stimulation for an individual β-blocking agent, however, the difference between the degree of metabolic and chronotropic blockade for a range of applied doses of β-blocking agents should be discussed (Fig. 2).

LB-46 blocked chronotropic and metabolic responses to IP in a linear, parallel dose-dependent manner.

PR inhibited in the same way chronotropic effect of IP only, while the blockade of phosphorylase activation occurred abruptly from zero level to 80 per cent of the maximal blockade when the dose of PR was doubled. Further 10-fold increase of dosage of PR did not augment the degree of inhibition of phosphorylase activation. This suggests that PR cannot induce the full blockade of metabolic effect of β-stimulation.

INPEA resulted a reverse picture to PR. Phosphorylase activation was blocked linearly while chronotropic effect of IP disappeared suddenly by 2-fold increase of dosage of INPEA.

In our previous paper we measured the blockade by PR and INPEA of the IP-induced phosphorylase activity and inotropic effect in isolated perfused rat hearts.[9] Then it seemed that there exist the preponderance of metabolic blockade by PR and the predominance of mechanical blockade by INPEA. This discrepancy between the results obtained *in vivo* and *in vitro* suggests that not only quantitative but also qualitative differences are found for β-blocking agents depending on the kind of technique used. In our experiments *in vivo* INPEA was only seven times less potent than PR. Similar results were obtained by Somani,[26] while in experiments *in vitro* INPEA was usually 100 times less potent than PR.[9,20,22,53] Similarly LB-46 was found to be 60 times more potent than PR in intact rats (this paper) and 10–40 times more potent in other's experiments *in vivo*,[19,27,48] while *in vitro* it was only 4–10 times more effective than PR.[19,27]

The above results may suggest that the class of β-blocking agents is not uniform and that there is the possibility of finding a drug which would block selectively, in a wide range of doses, metabolic or mechanical response of the heart to β-adrenergic stimulation.

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