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A potent β-adrenoreceptor blocking drug: 4-(2-hydroxy-3-isopropylaminopropoxy)indole

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4-(2-Hydroxy-3-isopropylaminopropoxy)indole (LB46) is a competitive β-adrenoreceptor blocking drug with a potency of between 4 and 7 times that of propranolol on the guinea-pig isolated trachea and atria (chronotropic effects). LB46 itself produces tracheal relaxation which may result from an indirect sympathomimetic action. The influence of uptake into adrenergic nerves on pA_2 and pA_{10} values for LB46 and propranolol, when using noradrenaline as agonist drug, has been assessed from results obtained on trachea in the presence and absence of cocaine (10^{-5}M). In the absence of cocaine the slopes of the regression of log (dose ratio −1) against negative log molar concentration of antagonist were less than the theoretical value of −1·0. In the presence of cocaine the slopes of these regressions approached −1·0. Thus values of (pA_2–pA_{10}) also deviated from the theoretical value in the absence of cocaine but approached it if cocaine was present in the bath fluid.

It has been suggested that β-adrenoreceptors might be of at least two types which have been called β_1 and β_2 (Lands & Brown, 1964; Lands, Arnold & others, 1967; Lands, Luduena & Buzzo, 1967; Furchgott, 1967). The β_1-adrenoreceptors include those initiating increased force and rate of contraction of the heart, lipolysis and inhibition of intestine. The β_2-adrenoreceptors include those initiating vasodilatation, bronchodilation and uterine relaxation. There has thus been some interest in the possibility of developing agonist and antagonist drugs specific to certain β-adrenoreceptor sites. Salbutamol has recently been described as a specific agonist of β-adrenoreceptors in the respiratory tract (Cullum, Farmer & others, 1968) whilst ICI 50 172 has been described as a specific antagonist of β-adrenoreceptors in the heart (Dunlop & Shanks, 1968). We have examined the β-adrenoreceptor blocking action of 4-(2-hydroxy-3-isopropylaminopropoxy)indole (LB46, prinodolol). The actions of LB46 have been compared with those of propranolol on both trachea and atria of guinea-pigs, as examples of tissues containing β_2 and β_1 adrenoreceptors respectively.

EXPERIMENTAL

Guinea-pig isolated tracheal chain

Relaxations of guinea-pig tracheal chains, prepared from adult female guinea-pigs, were recorded as described by Chahl & O'Donnell (1967). The preparations were suspended in Krebs bicarbonate solution containing ascorbic acid (200 μg/ml) and the cartilaginous part of each ring was cut. Cumulative dose-response curves to isoprenaline or noradrenaline were obtained using the method of van Rossum (1963).

Guinea-pig isolated atria

Isolated atria from adult female guinea-pigs were mounted in Krebs bicarbonate solution containing 200 μg/ml ascorbic acid and aerated with 5% carbon dioxide

in oxygen at 31°. The positive chronotropic effects of isoprenaline were recorded using a post office counter activated by a mercury contact. The resting atrial rate per min (i.e. the rate in the absence of agonist) was found by doubling the average of three 30 s counts taken at 1 min intervals. Doses of isoprenaline were then added using the cumulative method of Rossum (1963). After each dose, 1 min was allowed to elapse and then three measurements of the atrial rate were made over the next 3 min. The resulting 4 min interval between successive doses of agonist was adequate for the full effect of each dose to be observed. The response to each dose was taken as the resultant increase in atrial rate (i.e. highest atrial rate observed after each dose minus the resting atrial rate). This was then expressed as a percentage of the maximum increase in rate which could be induced by the agonist.

Experimental designs

On both trachea and atria two series of experiments were made using isoprenaline as the agonist drug. In one series LB46 was the antagonist drug and in the other propranolol. After obtaining a control dose-response line to isoprenaline, successive doses of either LB46 (0·002, 0·02, 0·2 and 2·0 μg/ml bath fluid) or propranolol (0·02, 0·2, 2·0 and 20·0 μg/ml bath fluid) were added to the bath. The effect of the antagonist on the responses to isoprenaline was tested 30 min after adding the dose of antagonist.

Further experiments were made on trachea using noradrenaline as the agonist drug, and the effect of doses of LB46 or propranolol was examined as described above. Experiments were then carried out where control dose-response lines to noradrenaline were obtained in the absence, and then in the presence, of cocaine (10^{-5}M) added to the bath fluid for 30 min in order to block uptake of noradrenaline into adrenergic nerve terminals. The effect on the responses to noradrenaline of successive doses of LB46 or propranolol in the presence of 10^{-5}M cocaine was then tested.

Determination of pA values and potency ratios

pA values for the antagonist drugs were obtained by a method based on that of Arunlakshana & Schild (1959). The ED50 value (concentration of agonist in the bath required to produce a 50% maximum response) was interpolated from each log dose—% maximum response line fitted by eye. Log (dose ratio–1) for the agonist was then plotted against the negative value of the log molar concentration of antagonist. Dose ratio is the ratio of the ED50 values for the agonist drug obtained in the presence and in the absence of antagonist. Results from all experiments in a series were collected and the best line through the points was calculated by a linear least squares regression. The pA_2 value (value of the intercept on the abcissa) was calculated from the values for the slope of the plot and the intercept on the ordinate. The pA_{10} value (the negative log molar concentration when log (dose ratio–1) equalled 0·95) was also calculated by substitution in the equation for a straight line, $y = mx + C$.

The potency of LB46 relative to that of propranolol was calculated by taking the antilog of the difference in the pA_2 or pA_{10} values.

Drugs

Drugs used were: cocaine hydrochloride, (±)-isoprenaline sulphate; LB46; (−)-noradrenaline acid tartrate; (±)-propranolol hydrochloride. LB46 was provided in ampoules containing 200 μg/ml LB46 as base with a small amount of citric acid in each ampoule. Therefore propranolol solutions were also prepared as μg/ml, expressed as weight of base. The doses of antagonist were converted to molar concentrations for calculation of pA values. Doses of all other drugs are expressed as final molar concentration in the bath fluid.

RESULTS

Direct effects of LB46 and propranolol on trachea and atria

Propranolol (0·02, 0·2 and 2·0 μg/ml) had little effect on the resting tone of tracheal muscle. An increase in tone was observed after 20·0 μg/ml propranolol. In most experiments LB46 (0·002, 0·02, 0·2 and 2·0 μg/ml) produced a loss of tone which was most marked on addition of the first dose (Fig. 1). Addition of cocaine alone to the bath caused a loss of tone of tracheal muscle, which was particularly marked when noradrenaline was the agonist. Cocaine in the presence of either of the β-adrenoreceptor blocking drugs caused no loss of tone.

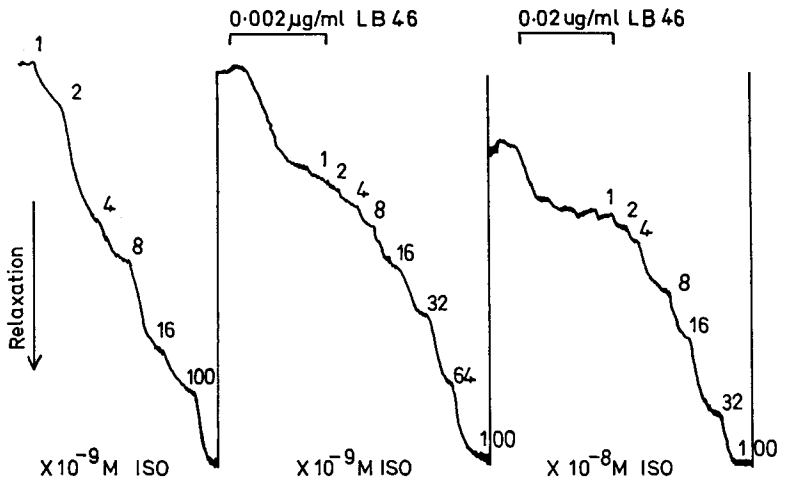

FIG. 1. Isoprenaline antagonism by LB46 on guinea-pig trachea, showing loss of tone of preparation on addition of LB46.

The effects of both LB46 and propranolol on the resting atrial rate after 30 min contact with the tissue are indicated in Table 1. Increasing doses of propranolol caused a marked depression of atrial rate. LB46 also depressed atrial rate but not as much as did propranolol.

Antagonism of isoprenaline by LB46 on trachea and atria

pA_2 and pA_{10} values for LB46 were compared with the values for propranolol on both trachea and atria using isoprenaline as agonist drug. For pA values to be valid it is necessary that the log dose—% maximum response line for the agonist be shifted by the antagonist in a parallel fashion to a higher dose range, i.e. the

Table 1. *Resting atrial rate (%) after 30 min contact of tissue with LB46 or propranolol*

Dose of blocking drug (μg/ml)	% resting atrial rate ± s.d.* LB 46	Propranolol
0·002	87·9 ± 9·9 (6)†	—
0·02	66·8 ± 23·1 (3)	83·9 ± 7·5 (5)
0·2	74·9 ± 21·7 (5)	78·0 ± 10·9 (5)
2·0	80·3 ± 8·6 (5)	65·9 ± 11·7 (5)
20·0	—	24·59 (2)‡

* Standard deviation.
† Number of observations.
‡ In 3 other experiments beating stopped.

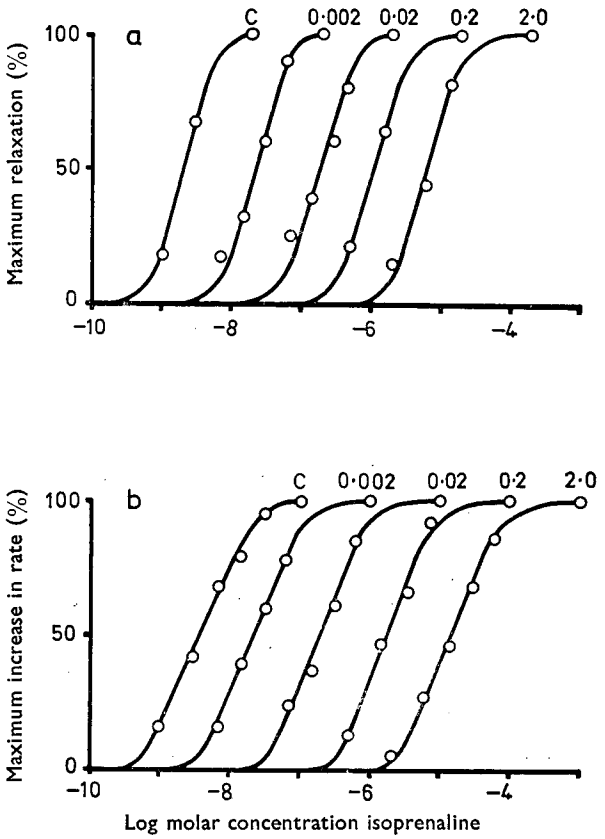

FIG. 2. Log dose–% maximum response lines to isoprenaline on (a) guinea-pig trachea and (b) guinea pig atria. As the dose of LB46 (0·002, 0·02, 0·2 and 2·0 μg/ml bath fluid) was increased the lines to isoprenaline were shifted parallel to the control line (C) suggesting a competitive antagonism.

antagonism must be competitive (Gaddum, 1957). Experiments illustrating this for LB46 against isoprenaline on both trachea and atria are shown in Fig. 2. There was no depression of the maximum response to isoprenaline by LB46, over the dose range used, confirming the competitive nature of the antagonism.

The regression lines for the values of log (dose ratio–1) plotted against the negative log molar concentration of antagonist and calculated from a number of experiments are illustrated in Fig. 3. The pA_2 and pA_{10} values obtained for propranolol on trachea were similar to those obtained on atria. Also, the values obtained for LB46 on trachea were similar to those on atria (Fig. 3). For the action of propranolol on both trachea and atria the slope of the line was in close agreement with −1·0 and therefore the pA_2–pA_{10} value (0·94 trachea, 0·91 atria) did not deviate markedly from 0·95, the theoretical value for a competitive antagonism. Values for the slope and pA_2–pA_{10} (0·91) for the action of LB46 on atria were also close to theoretical. However on trachea the slope was less than expected (Fig. 3) and the (pA_2–pA_{10}) value was therefore high (1·10).

The potency of LB46 relative to propranolol calculated from pA_2 values was 6·2:1 on trachea and 4·2:1 on atria. The potency ratios calculated from pA_{10} values were 4·3:1 and 4·2:1 respectively.

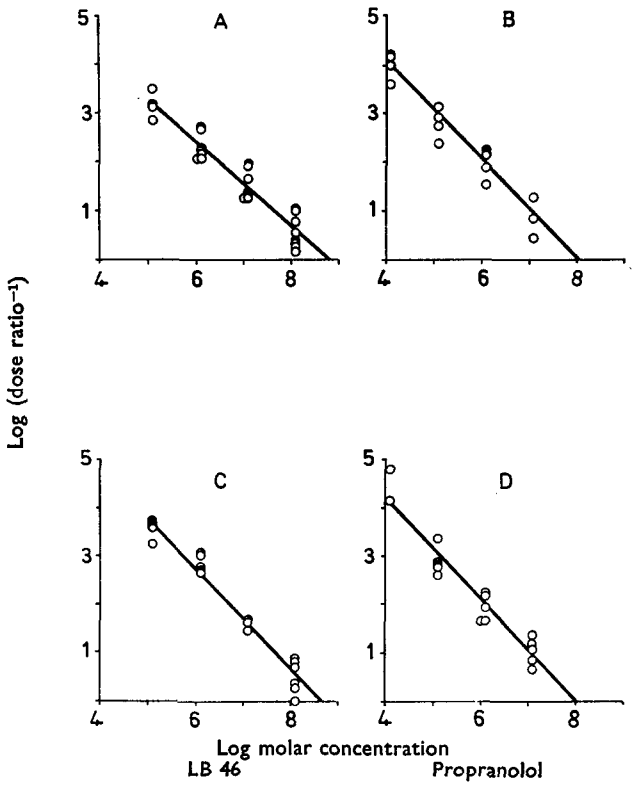

FIG. 3. Regression lines summarizing the results from 8 separate experiments using LB46 and 5 experiments using propranolol on both trachea (A, B) and atria (C, D), with isoprenaline as the agonist drug. Shows linear relation between log (dose ratio–1) for isoprenaline (ordinate) and negative value of log molar concentration of antagonist drug (abscissa). pA_2, pA_{10} and slope values are respectively: A, 8·81, 7·71, —0·86; B, 8·02, 7·08, —1·01; C, 8·64, 7·73, —1·04; D, 8·02, 7·11, —1·05.

Use of noradrenaline as agonist drug

It has been suggested that the observed response to some sympathomimetic amines is affected if that amine is taken up by adrenergic nerve terminals in a sympathetically-innervated isolated tissue preparation (Iversen, 1967). This has been studied experimentally on isolated trachea (Chahl & O'Donnell, 1967; Foster, 1967) and on isolated atria (Blinks, 1967). It was of interest to examine the effect of uptake of the agonist into adrenergic nerves on the pA_2 and pA_{10} values for antagonists. Thus the effects of LB46 and propranolol on the responses of trachea to noradrenaline in the absence and in the presence of cocaine were investigated. It was assumed that the dose of cocaine used (10^{-5}M) was sufficient to block most of the uptake of the amine into adrenergic nerve terminals in this tissue. The results obtained are summarized in Fig. 4. In the absence of cocaine the slopes of the regression of log (dose ratio–1) against negative log molar concentration of antagonist for both LB46 and propranolol were smaller when compared to the equivalent plots for isoprenaline as agonist. In the presence of cocaine the slopes of these regressions approached the theoretical value of −1·0 (see Fig. 4). Consequently, values of pA_2–pA_{10} (1·47 LB46; 1·23 propranolol) deviated from the theoretical value of 0·95

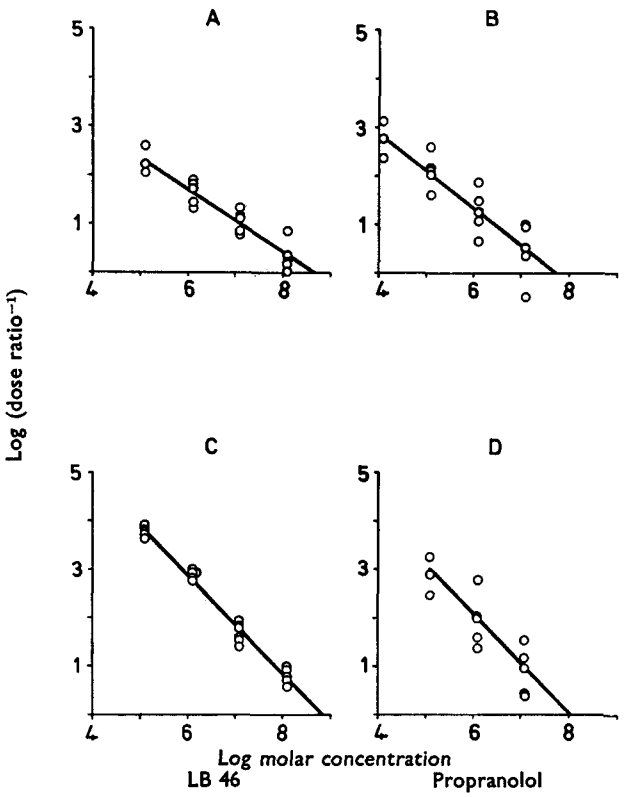

FIG. 4. Regression lines summarizing the results from separate experiments where LB46 or propranolol antagonized the noradrenaline relaxations of trachea in the absence (A, B) or presence (C, D) of cocaine (10^{-5}M). Log (dose ratio–1) for noradrenaline is plotted against negative value of log molar concentration of LB46 or propranolol. pA_2, pA_{10}, and slope values are respectively: A, 8·65, 7·18, —0·64 (5 exp.); B, 7·71, 6·48, —0·77 (5 exp.); C, 8·83, 7·91, —1·02 (6 exp.); D, 8·03, 7·08, —1·00 (5 exp.).

in the absence of cocaine but approached it if cocaine was present in the bath fluid (0·92 LB46; 0·95 propranolol). The potency of LB46 relative to propranolol on the trachea was calculated from the experiments using noradrenaline in the presence of cocaine. The potency ratio was 6·3 : 1 when calculated from pA_2 values and 6·8 : 1 from pA_{10} values.

DISCUSSION

Both propranolol and LB46 fulfilled the various criteria necessary for competitive antagonism of β-adrenoreceptors on guinea-pig trachea and atria. They produced a parallel shift in the log dose—% maximum response lines to isoprenaline and there was no depression of the maximum response to isoprenaline after high doses of antagonist drug. When the results of these experiments using isoprenaline were plotted as log (dose ratio–1) against the negative log molar concentration of antagonist the results were fitted by a straight line. The slopes of these regressions approached the theoretical value of −1·0, and (pA_2–pA_{10}) values approached the theoretical value of 0·95 for competitive antagonism except in those experiments on trachea where the antagonism of isoprenaline by LB46 was studied. In these experiments the relatively low slope value (0·86) resulted in an increase in the value of (pA_2–pA_{10}) to 1·10. In experiments on trachea where noradrenaline was used as agonist drug, the slopes of the regression lines were also less than the theoretical value of −1·0 and consequently the values for (pA_2–pA_{10}) were high. These latter deviations from theoretical values were considered to be due to loss of noradrenaline into adrenergic nerves since, in experiments where cocaine (10^{-5}M) was also present, the values for both the slope and (pA_2–pA_{10}) were close to theoretical. Thus the pA_2 value of 6·56 for propranolol on trachea quoted by Foster (1966) is probably too low since noradrenaline was used as the agonist drug and its loss by uptake into adrenergic nerves was not prevented.

Various alterations in the resting tone of tracheal preparations were observed after addition of some of the drugs. Propranolol had little effect on resting tone except for the 20·0 μg/ml dose where an increase in tone occurred. This finding agrees with that of Foster (1966) but is in contrast to the observations of Åblad, Brogård & Ek (1967) who describe relaxations of guinea-pig trachea with similar doses of propranolol. However, they used pilocarpine to induce tone and propranolol might therefore have relaxed the tissues by an antimuscarinic action (Mazurkiewicz, 1968). Cocaine or LB46 alone both caused loss of tone of the preparations. The loss of tone by cocaine was particularly marked when noradrenaline was used as agonist drug. It is possible that a spontaneous release of noradrenaline influences the resting tone of individual tracheal preparations and that cocaine blocks the re-uptake of this spontaneously released noradrenaline. The addition of cocaine together with either LB46 or propranolol caused no loss of tone. This might be because the β-adrenoreceptor blocking drugs occupy the β-adrenoreceptors necessary for cocaine-induced relaxation. The loss of tone by LB46 alone was particularly marked on addition of the first dose of drug (usually the 0·002 μg/ml dose) and then it was only slightly increased as LB46 was added again. The absence of this relaxation of the tissue by LB46 in the presence of cocaine (which itself induces loss of tone) might suggest that LB46 is an indirect sympathomimetic amine and that its access to noradrenaline storage sites is prevented by the simultaneous presence of cocaine. The loss of tone was considered not to

be due to local anaesthetic activity since LB46 and propranolol are approximately equipotent as local anaesthetics (unpublished results) but propranolol does not depress the tone of the tissue. If the relaxation of the muscle by LB46 were a non-specific effect, cocaine, by a summation effect, would probably exaggerate and not prevent the relaxation.

It is possible that the loss of tone of tracheal preparations following the addition of LB46 might account for the reduced slope of the regression of log (dose ratio–1) against negative log molar concentration LB46 observed using isoprenaline on trachea. The slope of the regression was not reduced under similar conditions when propranolol was the antagonist. It was assumed that the reduction of slope was not related to uptake of agonist into nerves, since isoprenaline has little affinity for this uptake (Hertting, 1964; Iversen, 1967).

Both propranolol and LB46 caused some depression of resting atrial rate. The effects of LB46 were not as marked as those of propranolol, but, if LB46 has some indirect sympathomimetic action, this could cause a physiological antagonism of any direct depressant effects of LB46 on atrial rate. Our findings with propranolol confirm those of Blinks (1967) who has previously described the negative chronotropic effects on guinea-pig atria of propranolol at doses greater than 10^{-6}M with atrial arrest with 10^{-4}M propranolol.

If the receptors for an agonist drug are the same in different tissues then the pA_2 and pA_{10} values for an antagonist of these receptors in those tissues should be the same (Arunlakshana & Schild, 1959). The pA_2 and pA_{10} values for propranolol against isoprenaline were the same on trachea and atria. The pA_2 and pA_{10} values for LB46 against isoprenaline were also similar on trachea and atria.

The pA_2 or pA_{10} values were used to compare the potency of LB46 and propranolol as β-adrenoreceptor blocking drugs on these two tissues. If the slopes of the regression lines agree closely with the theroetical value of $-1{\cdot}0$ then the potency ratio should be similar using either the pA_2 or the pA_{10} values. However, at least two factors in the experiments described might affect the slope, viz. uptake into adrenergic nerves and direct effects of the antagonist on the tissue. Thus there was some variation in potency ratio depending upon the experimental conditions. Nevertheless, LB46 was from 4 to 7 times more potent than propranolol and the potency ratios on trachea and atria could not be considered to be markedly different.

The value of the pA_2 found in this work for propranolol antagonism of the chronotropic effects of isoprenaline (8·02) was less than the value (8·56) reported by Blinks (1967). This could merely reflect the wide variability in pA_2 values which occurs from experiment to experiment when using adrenoreceptor blocking drugs. Alternatively, it could be because we used a ten fold higher dose of propranolol than Blinks and other actions of either the antagonist or the agonist might interfere. It is also possible that complete β-adrenoreceptor blockade was not attained after a 30 min contact time of the tissue with propranolol. To examine 3 or 4 doses of propranolol on one tissue, we were not able to use a longer contact time.

It has thus been concluded that LB46 is a more potent β-adrenoreceptor blocking drug than propranolol on guinea-pig trachea and atria and is not specific for either tissue. It may have sympathomimetic actions which are manifest as a relaxation of guinea-pig trachea and which might also obscure direct negative chronotropic effects of LB46 on guinea-pig atria. pA_2 and pA_{10} values have been used to estimate the potency ratio of the two β-adrenoreceptor blocking drugs but it is felt that

many factors, such as adrenergic uptake and direct effects of the blocking drug on the tissue, might influence these values and reduce their reliability.

Since the completion of the work described in this paper two reports on LB46 have been published. Giudicelli, Schmitt & Boissier (1969) found LB46 to be 5–7 times more potent than propranolol on guinea-pig trachea and atria (inotropic effects) using isoprenaline as agonist. Lubawski & Wale (1969) found LB46 to be 10 times more potent than propranolol on rabbit atria (chronotropic effects) but these authors used pA_2 values obtained using adrenaline as agonist drug which may introduce a variance due to slight loss of adrenaline into adrenergic nerves. The experiments of Lubawski & Wale (1969) indicated that LB46 might have weak β-receptor agonist activity whereas Giudicelli & others (1969) consider that it has no intrinsic β-sympathomimetic activity.

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