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AN INVESTIGATION OF THE β-ADRENOCEPTOR THAT MEDIATES METABOLIC RESPONSES TO THE NOVEL AGONIST BRL28410 IN RAT SOLEUS MUSCLE

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Abstract—The β-adrenoceptor agonist BRL26830A selectively stimulates metabolic rate in the rat and this thermic effect is resistant to blockade by propranolol. These effects of BRL26830A are partly due to selective stimulation by its metabolite BRL28410, of brown adipocyte β-adrenoceptors, these receptors being resistant to propranolol. To investigate whether the metabolic effects of BRL28410 in skeletal muscle are also mediated by atypical β-adrenoceptors, the potencies of BRL28410 and isoprenaline were compared for β-adrenoceptor mediated responses in rat stripped soleus muscle. In addition, pA_2 values for antagonism by propranolol of these responses were determined. Isoprenaline had similar EC_{50} values for stimulation of lactate formation (4.3×10^{-9} M) and inhibition of glycogen synthesis (3.4×10^{-9} M) and these values are similar to its reported EC_{50} values for stimulation of atrial rate (β_1-adrenoceptor-mediated) and relaxation of the uterus (β_2-adrenoceptor-mediated). BRL28410 had similar EC_{50} values for stimulation of lactate formation (3.7×10^{-6} M) and inhibition of glycogen synthesis (3.8×10^{-6} M). These values are only about two-fold less than reported values for relaxation of the uterus, but ten-fold less than reported values for stimulation of atrial rate. The pA_2 value of *dl*-propranolol for antagonism of the effect of isoprenaline on glycogen synthesis (8.38 ± 0.13) was in the range expected for β_1- or β_2-adrenoceptors, but with BRL28410 as agonist the pA_2 value was about one unit lower (7.39 ± 0.11). The β-adrenoceptors that mediate the metabolic effects of BRL28410 in soleus muscle therefore differ from those that mediate atrial rat, uterine relaxation and adipocyte lipolysis. In addition, the low pA_2 value of *dl*-propranolol versus BRL28410 in rat soleus muscle, which contrasts with the normal pA_2 value previously reported for guinea-pig trachea, suggests that β_2-adrenoceptors in these two tissues can be differentiated with suitable pharmacological agents.

The novel β-adrenoceptor agonist BRL26830A reduces lipid accumulation or promotes lipid loss in obese rats and mice by stimulating metabolic rate, rather than by reducing food intake [1–3]. The biological effects of orally administered BRL26830A are mediated *in vivo* by its de-esterified metabolite BRL28410 (BRL26830A is not found in the systemic circulation and no other compounds with pharmacological activity have been identified: P. J. Baines and G. Mellows, unpublished results). Studies in pithed rats have shown that BRL28410 selectively stimulates an atypical class of β-adrenoceptors that promote thermogenesis and has lower potency at the typical β_1- and β_2-adrenoceptors which mediate the cardiac and vascular effects of β-adrenoceptor agonists [4]. Studies *in vitro* have shown that BRL28410 stimulates an atypical receptor in brown and white adipose tissue. Thus it is more potent as a stimulant of adipocyte lipolysis than responses mediated by β_1- (atrial rate) or β_2- (uterine and bronchial relaxation) adrenoceptors [2, 3, 5]. Further evidence that BRL28410 interacts with atypical β-adrenoceptors is provided by the findings that unusually high concentrations of propranolol are required to inhibit the thermic effect of BRL26830A *in vivo* [1] and BRL28410-stimulated lipolysis in brown and white adipocytes *in vitro* [3, 5].

Since skeletal muscle may play an important role in mediating the effects of BRL26830A on metabolism [6], it was of interest to see whether responses mediated by β-adrenoceptors show similar characteristics in rat skeletal muscle to those found previously in adipocytes. The responses selected for study were inhibition of glycogen synthesis and stimulation of lactate formation in isolated stripped soleus muscle [7].

MATERIALS AND METHODS

Rat soleus strips were prepared from male Wistar rats (160–180 g) as described previously [7, 8]. This involved longitudinal section of the muscle, ligature of distal and proximal tendons and attachment of the strip to a stainless steel spring to maintain the muscle at resting tension. After a 30 min pre-incubation period [8], muscles were incubated singly at 37° in Krebs–Ringer bicarbonate buffer, pH 7.4 containing 1% (w/v) defatted bovine serum albumin, 5 mM glucose (containing approximately 0.25 μCi of [U-^{14}C] glucose/ml), 0.5 nM insulin and appropriate

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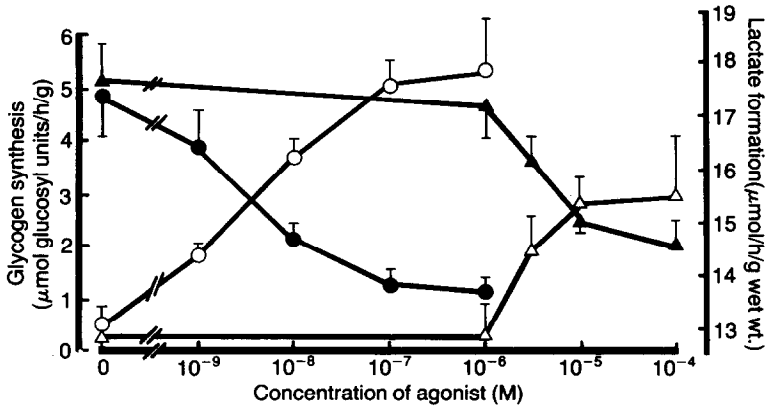

Fig. 1. Effects of BRL28410 (▲, △) and isoprenaline (●, ○) on glycogen synthesis (closed symbols) and lactate formation (open symbols), in rat isolated stripped soleus muscle. Each point is a mean of mean values from five (BRL28410) or six (isoprenaline) experiments with a bar for SE (calculated from SD using $n = 5$ or 6).

concentrations of β-adrenoceptor agonists and antagonists as described. The incubation period was 60 min in a shaking water bath (120 oscillations/min), after which the muscle strips were removed, rapidly blotted and freeze-clamped. Lactate concentration in the incubation medium was measured spectrophotometrically [9], and [U-^{14}C]glucose incorporation into glycogen was measured following alkaline digestion of the freeze-clamped muscle and ethanolic precipitation of glycogen as described previously [10].

Concentration–response curves were constructed in six independent experiments for isoprenaline and five further independent experiments for BRL28410 using the concentrations of agonist indicated in Fig. 1. Preliminary experiments showed that increases in isoprenaline concentration above 10^{-6} M, and BRL28410 concentration above 10^{-4} M had no greater effects on the rates of lactate formation or glycogen synthesis in the stripped soleus muscle preparation. In each experiment, each concentration of agonist was replicated at least four times. EC_{50} values were determined for each concentration–response curve using the minimum and maximum rates of lactate formation or glycogen synthesis of each curve as the 0 and 100% points. Geometric mean EC_{50} values with 95% confidence limits were calculated from these EC_{50} values.

The relative intrinsic activity (RIA) of BRL28410 was determined for each BRL28410 concentration–response curve taking the mean maximum effect of isoprenaline as 1.0. The arithmetic means of these RIA values are given in Table 1.

For the determination of pA_2 values for *dl*-propranolol, concentration–response curves to isoprenaline or BRL28410 were constructed in the presence of two concentrations of *dl*-propranolol used (with the number of experimental repeats given in parentheses) were 6×10^{-8} (1), 2×10^{-7} (1), 6×10^{-7} (2) and 2×10^{-6} (1) M for isoprenaline and 2×10^{-7} (3), 6×10^{-7} (2), 2×10^{-6} (2) and 6×10^{-6} (1) M for BRL28410. pA_2 values were determined by method (b) of MacKay [11] in which a pA_2 value is calculated for each concentration–response curve

Table 1. Mean EC_{50} and RIA values for stimulation of lactate formation and inhibition of glycogen synthesis by isoprenaline and BRL28410 in rat isolated stripped soleus muscle

	Isoprenaline	BRL28410	
Activity	EC_{50} (M)	EC_{50} (M)	RIA
Lactate formation	4.3×10^{-9} (1.2–15.5)	3.7×10^{-6} (1.4–9.8)	0.56 ±0.08
Glycogen synthesis	3.4×10^{-9} (1.8–6.5)	3.8×10^{-6} (3.2–5.6)	0.87 ±0.05
Atrial rate stimulation	1.3×10^{-9}	3.7×10^{-5}	0.69
Uterine relaxation	3.1×10^{-9}	8.2×10^{-6}	0.82

Values are means of six (isoprenaline) or five (BRL28410) experiments. EC_{50} values are geometric means with 95% confidence limits in parenthesis (same exponent as the mean). RIA values are arithmetic means ± SE. The RIA for isoprenaline is set as 1.0. Values for stimulation of rat right atrial rate and relaxation of uterine tension are taken from Ref. 5. All EC_{50} values are relative to the compound's own maximum effect.

Table 2. Potencies of isoprenaline and BRL28410 for lactate formation stimulation and glycogen synthesis inhibition relative to atrial rate stimulation and uterine relaxation

Potency ratio	Isoprenaline	BRL28410	Potency ratio BRL28410 / Potency ratio isoprenaline
Lactate: atria	0.30	10.0	33.3
Glycogen: atria	0.38	9.7	25.6
Lactate: uterus	0.72	2.2	3.1
Glycogen: uterus	0.91	2.2	2.4

in the presence of *dl*-propranolol by comparing it with its respective control curve.

RESULTS

Concentration–response curves for stimulation of lactate formation and inhibition of glycogen synthesis by BRL28410 and isoprenaline are shown in Fig. 1 in the form of combined results for all the experiments. EC_{50} and RIA values were determined in each experiment and the mean of these values are shown in Table 1. Table 1 also shows for comparison the EC_{50} and RIA values for stimulation of rat right atrial rate and relaxation of the uterus by isoprenaline and BRL28410 (from Ref. 5).

Isoprenaline had very similar potencies for stimulation of lactate formation and inhibition of glycogen synthesis in soleus muscle. These potencies were also similar to its potencies as a stimulant of atrial rate and relaxant of the uterus. BRL28410 was about a thousand-fold less potent than isoprenaline in soleus muscle and was a partial agonist for both effects. Like isoprenaline, BRL28410 had very similar potencies for stimulation of lactate formation and inhibition of glycogen synthesis, and these potencies were similar to its potency as a uterine relaxant. However, unlike isoprenaline, BRL28410 was more potent in soleus muscle than as a stimulant of atrial rate. This difference in the selectivities of BRL28410 and isoprenaline is further illustrated in Table 2 in which selectivities are expressed as reciprocals of the ratios of EC_{50} values. There is a difference of about thirty-fold between BRL28410 and isoprenaline in the potency ratios of the soleus muscle effects relative to the β_1-adrenoceptor-mediated effect (atrial rate), whereas the difference is only two- to three-fold in the potency ratios of the soleus muscle effects relative to the β_2-adrenoceptor-mediated effect (uterine relaxation).

Propranolol caused parallel shifts to the right in the concentration–response curves for inhibition of glycogen synthesis by isoprenaline and BRL28410. The pA_2 value for antagonism of the effect of isoprenaline showed no significant regression on log [propranolol]. The mean value of 8.38 (Table 3) was in the range expected for antagonism of typical β_1- and β_2-adrenoceptors [5] and well above the values of 6.6 and 7.3 previously reported for antagonism of the lipolytic effect of isoprenaline in white [5] and brown [3] adipose tissue.

The pA_2 value for antagonism of the effect of BRL28410 on glycogen synthesis (Table 3) was higher at the lower concentrations of propranolol (2×10^{-7}, 6×10^{-7} M) than at the higher concentrations (2×10^{-6}, 6×10^{-6} M) with the result that there was a significant regression pA_2 on log [propranolol] ($r = 0.67$; $P < 0.05$). This is equivalent to the slope of a Schild plot [12] being less than 1. The mean pA_2 value for the lower concentrations of propranolol was 7.58 ± 0.10 (5) which is significantly less ($P < 0.01$, unpaired Student's *t*-test) than the pA_2 value with isoprenaline as agonist and also less than the pA_2 values for antagonism by propranolol of the effects of BRL28410 on atrial tension (β_1-adrenoceptor-mediated, $pA_2 = 8.7$) and tracheal tension (β_2-adrenoceptor-mediated, $pA_2 = 8.6$) [5]. However, the value of 7.58 is not as low as the values of 6.2 and 6.8 previously reported for antagonism of the lipolytic effect of BRL28410 in white [5] and brown [3] adipose tissue.

The pA_2 values for antagonism by propranolol of the effects of isoprenaline and BRL28410 on lactate formation are not shown because there was poor agreement between replicate measurements in a number of experiments, and parallel shifts in the concentration–response curves were not always found. However, those pA_2 values that were obtained were again higher when isoprenaline, rather than BRL28410, was the agonist (results not shown).

Table 3. pA_2 values for antagonism by propranolol of the effects of isoprenaline and BRL28410 on glycogen synthesis in soleus muscle

Concentration or propranolol (M)	Agonist: Isoprenaline	BRL28410
6×10^{-8}	8.19	—
2×10^{-7}	8.13	7.33, 7.54, 7.81
6×10^{-7}	8.16, 8.73	7.43, 7.81
2×10^{-6}	8.67	6.98, 7.04
6×10^{-6}	—	7.19
Mean ± SE	8.38 ± 0.13	7.39 ± 0.11

DISCUSSION

In studying the nature of the β-adrenoceptor stimulated by BRL28410 in skeletal muscle, we did not choose to measure the binding of agonists and antagonists to skeletal muscle β-adrenoceptors because our unpublished work has shown that the selective lipolytic effect of BRL28410 in white adipocytes is not due to selective binding to white adipocyte β-adrenoceptors (P. Young and J. R. S. Arch, unpublished results). Furthermore, the low pA_2 values of propranolol for inhibition of lipolysis in rat brown [3, 13] and white [5, 14] adipocytes are not consistent with its high binding affinity for adipocyte β-adrenoceptors [15], and this has led to the hypothesis that the receptors detected by binding are not those that mediate the functional response, i.e. lipolysis [15, 16]. In addition, the selectivity of certain selective β-adrenoceptor agonists and antagonists for effects on tracheal and cardiac muscle cannot be explained in terms of selective binding [17]. The potencies of BRL28410, isoprenaline and propranolol in influencing a functional response in skeletal muscle were therefore studied.

The results show that the β-adrenoceptors that mediate the effects of BRL28410 on glycogen synthesis and lactate formation in soleus muscle differ from those that mediate atrial rate, uterine relaxation and adipocyte lipolysis. The soleus muscle receptor appeared most similar to the uterine receptor, consistent with the classification of both receptors as of the β_2-subtype. However, propranolol had a lower pA_2 value in soleus muscle than reported previously for uterine muscle when BRL28410 (but not isoprenaline) was the agonist. This low pA_2 value suggests that there is also some similarity between the soleus muscle receptor stimulated by BRL28410 and adipocyte β-adrenoceptors. The finding that propranolol had a higher pA_2 value when the agonist was isoprenaline rather than BRL28410 (Table 3) appears similar to findings for lipolysis in white [5] and brown [3] adipocytes, though in the adipocyte preparations the differences in pA_2 values were smaller (about 0.5 rather than 1 unit) and not significantly different. A further difference between adipocytes and soleus muscle was that the pA_2 value of propranolol for antagonism of isoprenaline-stimulated lipolysis was low, indicating that isoprenaline, like BRL28410, stimulates an atypical β-adrenoceptor in adipocytes. The difference in the propranolol pA_2 values for the two agonists suggests that they do not interact with identical receptors.

Since we know of no instance where different responses to an agonist in the same tissue are mediated by different β-adrenoceptors, it seems possible that heat production in soleus muscle is mediated by the same receptors that mediate glycogen synthesis and lactate formation. Thus it is possible that the selectivity of BRL26830A as a stimulant of thermogenesis rather than heart rate *in vivo*, and the low potency of propranolol as an antagonist of its thermic effect [1], is because BRL28410 stimulates thermogenesis in muscle [6] as well as brown adipose tissue [2].

Finally, it should be noted that the pA_2 value of propranolol versus BRL28410 appears lower in rat soleus muscle than in guinea-pig trachea (7.4 versus 8.6 [5], respectively). The significance of this difference to the classification of β_2-adrenoceptors may be questioned because the pA_2 value versus BRL28410 was not independent of the concentration of propranolol. Furthermore, it is possible that these different values reflect a species difference. Nevertheless, these results raise the possibility that β_2-adrenoceptors in the respiratory tract and skeletal muscle can be distinguished with suitable pharmacological agents. Therefore, it may yet be possible to develop a β_2-bronchodilator selective from tremorigenic activity [cf. 18, 19].

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