JPP Journal of Pharmacy and Pharmacology

JPP 2003, 55: 253–257

Received July 3, 2002
Accepted November 20, 2002
DOI 10.1211/002235702595
ISSN 0022-3573

Sensitivity to β-adrenoceptor agonists of adipocytes from rats treated with an aqueous extract of *Croton cajucara* Benth

Dora Maria Grassi-Kassisse, Valéria Wolf-Nunes, Alexandre Marcucci Miotto, Elisângela Farias-Silva, Alba Regina Monteiro Souza Brito, Domingos Savio Nunes and Regina Célia Spadari-Bratfisch

Abstract

Aqueous extracts of *Croton cajucara* bark are used in folk medicine to treat hepatic and gastrointestinal disorders and as a coadjuvant in weight-loss programs. We examined the effect of treating rats for 15 days with a 5% aqueous extract of *C. cajucara* on body weight and food intake. The epididymal adipose pads were removed and the lipolytic responses of isolated adipocytes to isoprenaline, noradrenaline (norepinephrine), BRL37344 and adrenaline (epinephrine) were analysed in the absence or presence of metoprolol or ICI118,551. Treated rats had a significantly lower weight gain than control rats, with no difference in food and liquid intake, epididymal fat-pad weight or basal glycerol release. The sensitivity of the lipolytic response to isoprenaline and adrenaline was significantly higher in adipocytes from treated rats. The sensitivity to noradrenaline or BRL37344 was unaltered. Metoprolol shifted the dose–response curves to noradrenaline to the right in adipocytes from control and treated rats; the dose–response curve to isoprenaline in adipocytes from control rats was also shifted to the right. In adipocytes from treated rats, the dose–response curve to isoprenaline was unaltered by metoprolol but was shifted to the right by ICI118,551, a β_2-adrenoceptor antagonist. We conclude that in adipocytes from treated rats there is an increase in the lipolytic response to non-selective agonists (isoprenaline and adrenaline) mediated by β_2-adrenoceptors, with no alteration in the responses mediated by β_1-adrenoceptors (noradrenaline) or β_3-adrenoceptors (BRL37344). This effect could increase the role of adrenaline as an endogenous stimulator of lipolysis.

Departamento de Fisiologia e Biofísica, Instituto de Biologia, Universidade Estadual de Campinas (UNICAMP), CP 6109, CEP 13083-970, Campinas, SP, Brasil

Dora Maria Grassi-Kassisse, Valéria Wolf-Nunes, Alexandre Marcucci Miotto, Elisângela Farias-Silva, Alba Regina Monteiro Souza Brito, Regina Célia Spadari-Bratfisch

Instituto de Química, Universidade Estadual de Ponta Grossa, CEP 84035-310, Ponta Grossa, PR, Brasil

Domingos Savio Nunes

Correspondence:
D. M. Grassi-Kassisse, Departamento de Fisiologia e Biofísica, Instituto de Biologia, Universidade Estadual de Campinas (UNICAMP), CP 6109, CEP 13083-970, Campinas, SP, Brasil.
E-mail: doramgk@unicamp.br

Funding: This work was supported by grants from FAPESP (96/4040-0, 96/4041-6, 98/00146-3).

Introduction

Croton cajucara Benth. (Euphorbiaceae) is a well-known medicinal plant used in Amazonian folk medicine to treat illnesses such as diarrhoea and diabetes, and to control high cholesterol levels. The stem bark of *C. cajucara* is prepared as a tea to be drunk in cases of heartburn, gastritis and peptic ulcer. Because of its strong bitter taste, the tea is taken daily in low doses for extended periods (2–8 weeks; Di Stasi et al 1994; Souza Brito & Nunes 1997). The infusion prepared with *C. cajucara* bark fragments contains *trans*-dehydrocrotonin (DHC), a bitter nor-clerodane diterpene, as the major component and an essential oil, containing primarily sesquiterpenes (Nunes et al, unpublished data). The anti-ulcerogenic mechanisms of DHC and the essential oil have been studied (Souza Brito et al 1998; Hiruma-Lima et al 1999a, b, 2002).

Recently, the use of *C. cajucara* as tea or as powdered and dried pills by overweight people has been recommended for slimming, but the prolonged use required has been correlated with frequent toxic hepatitis (Souza Brito & Nunes 1997; Maciel et al 1998, 2000). Unravelling the hormonal and neuroendocrine systems that regulate energy balance and body fat has been a long-standing challenge in biology, particularly with obesity as an increasingly important public health problem (Björntorp 1996). Adipose tissue is the body's largest energy reservoir. The primary role of adipocytes is to store triacylglycerol during periods of caloric excess and to mobilize this reserve when energy expenditure exceeds the intake. Mature adipocytes are uniquely equipped to

perform these functions since they possess the full complement of enzymes and regulatory proteins needed to carry out lipolysis and de-novo lipogenesis (Kopelman 1998; Lafontan & Berlan 1993, 1995).

In this work, we examined the effect of treating rats for 15 days with an aqueous extract of *C. cajucara* bark on body weight and food intake, as well as the sensitivity of the lipolytic responses to β-adrenoceptor agonists in isolated adipocytes.

Methods

Animals

Adult male Wistar rats (*Rattus norvegicus*), 326 ± 18 g ($n = 36$) at the beginning of the experiments, were used. The rats were housed in metabolic cages at 22 °C on a 12-h light–dark cycle, with lights on at 0630 h. The rats had access, daily, to 100 g of standard laboratory chow and 100 mL of liquid (water or *C. cajucara* extract). During the experiments, the rats were cared for in accordance with the principles outlined by Olfert et al (1993) for the use of animals for research and education, and the experimental protocols were approved by the Committee for Ethics in Animal Experimentation of the Institute of Biology (UNICAMP, 058-1).

Chemicals

Adenosine 5′-triphosphate, α-glycerophosphate dehydrogenase, β-nicotinamide adenine dinucleotide, bovine serum albumin (fraction V), collagenase (type II), glycerokinase, HEPES, (±)isoprenaline hydrochloride, (±)-metoprolol and (±)-noradrenaline (norepinephrine) were from Sigma Chemical Co. (St Louis, MO). BRL37344 and ICI118,551 were obtained from Tocris Cookson, Inc. (Ballwin, MO).

Plant material

Fragments of *C. cajucara* bark were collected on an experimental plantation in Benfica (Pará, Brazil). A voucher specimen was identified and deposited in the Museu Paraense Emílio Goeldi (accession number 247). The bark (5 g) was ground and mixed with boiling water (100 mL) to provide a 5% aqueous extract. After 20 min, the mixture was filtered through filter paper and the extract was offered to the rats instead of drinking water for 15 days. Chromatographic analysis (^{13}C-NMR) of a lyophilized sample showed that the extract contained 0.3 mg of *trans*-dehydrocrotonin (DHC, the major compound) per milliliter (Nunes, unpublished data).

Adipocyte preparation and the measurement of lipolysis

Control and treated rats were starved for 16 h before sacrifice. The rats were anaesthetized with sodium pentobarbital (60 mg kg^{-1}, i.p.) and the epididymal adipose tissue was removed. Lipolytic activity was analysed in isolated fat cells (Rodbell 1964). Krebs-Ringer bicarbonate buffer containing bovine serum albumin (3%), HEPES (0.238 g/100 mL) and glucose (6 mM), pH 7.4 (KRBA), was used. After incubation with collagenase (1 mg mL^{-1}, 45 min), isolated fat cells were filtered through a nylon mesh, washed and adjusted with KRBA to a 10% suspension. The cells were incubated with gentle shaking in vials containing 1 mL of KRBA plus pharmacological agents for 100 min (pre-incubation with antagonists for 40 min). After incubation, the tubes were placed in an ice bath, and samples of the infranatant were taken for the determination of glycerol (Weiland 1957), which was used as an indicator of lipolysis. The total lipid content was evaluated gravimetrically after extraction (Dole & Meinertz 1960). Concentration–response curves for agonists were obtained in the absence and presence of antagonist. Half-maximal effective drug concentrations (EC_{50}) were obtained and expressed as pD_2 values ($-\log EC_{50}$), with GraphPad Prism software (graphPad Software, Santiago, CA).

Statistical analysis

The values are shown as means ± s.e.m. and were analysed statistically using Student's *t*-test (paired or unpaired) for comparisons between pairs. Differences were considered significant for $P < 0.05$.

Results

The body weight of control rats (327 ± 23 g, $n = 12$) did not differ from that of treated rats (325 ± 11 g, $n = 24$) at the start of the experiment. Control rats showed a significant increase in body weight after 14 days ($+ 31.7 \pm 6.36$ g or 7% of the weight on day zero, $P < 0.05$) whereas treated rats showed no significant increase in body weight in this same period ($+ 13.5 \pm 7.9$ g or 3% of the weight on day zero). Starving for 16 h decreased the body weight when compared with the values on day 14, which were similar in both groups (not shown). The weight of the epididymal fat pad relative to the body weight was not significantly different between the two groups (control 1.24 ± 0.08%; treated 1.17 ± 0.08%). The mean daily food intake at the beginning of the experiment (control 30.6 ± 1.3; treated 32.6 ± 1.3 g/rat) was not different and remained similar up to the 14th day (control 31.2 ± 2.3; treated 31.7 ± 1.3 g/rat). Thus, treated rats did not gain weight, even though they consumed the same amount of food as the control rats. The daily liquid intake was also not different between the groups at the beginning of the treatment (control 42.4 ± 3.4; treated 49.9 ± 4.9 mL/rat) or after 14 days (control 41.4 ± 5.6; treated 41.2 ± 3.3 mL/rat). Since DHC was the main component of the *C. cajucara* aqueous extract (0.3 mg mL^{-1} of infusion), we estimated that the rats received a dose equivalent to 12.4–15.0 mg of DHC per day.

Table 1 Lipolytic potency and maximal lipolytic responses to β-adrenoceptor agonists in the absence or presence of β-adrenoceptor antagonists in adipocytes from rats treated with 5% aqueous extract of *Croton cajucara* bark compared with control rats.

	Control		n	Treated		n
	pD_2[a]	R_{max}[b]		pD_2[a]	R_{max}[b]	
Isoprenaline						
No antagonist	7.67 ± 0.18	1.84 ± 0.11	11	8.16 ± 0.12*	2.37 ± 0.32	9
+ Metoprolol (1 μM)	5.43 ± 0.19**	1.64 ± 0.18	3	8.16 ± 0.01	3.85 ± 0.71	3
+ ICI118,551 (50 nM)	7.56 ± 0.12	1.74 ± 0.10	6	7.87 ± 0.20**	1.47 ± 0.19	5
Noradrenaline						
No antagonist	6.87 ± 0.07	1.62 ± 0.17	8	6.96 ± 0.11	1.55 ± 0.18	9
+ Metoprolol (1 μM)	6.68 ± 0.14**	1.69 ± 0.29	4	6.73 ± 0.16**	1.89 ± 0.14	7
+ ICI118,551 (50 nM)	6.76 ± 0.08	1.67 ± 0.12	6	6.98 ± 0.08	1.14 ± 0.17	5
Adrenaline	5.71 ± 0.12	1.52 ± 0.11	7	6.68 ± 0.27*	1.78 ± 0.25	8
BRL37344	8.91 ± 0.19	1.42 ± 0.26	7	8.99 ± 0.22	1.53 ± 0.11	9

The values are the means ± s.e.m. of the number of experiments (n) done in duplicate. [a]The potencies of the lipolytic agents were compared based on their EC_{50} values (concentration of agonist causing 50% of maximum lipolysis), expressed as pD_2 ($-\log EC_{50}$). [b]Maximum response to the agonist, expressed in μmol of glycerol released/100 min/100 mg of total lipids. *$P < 0.05$, compared with the control values; **$P < 0.05$, compared with the same group without antagonist; Student's paired or unpaired *t*-test.

The responsiveness to β-adrenoceptor agonists was assessed in adipocytes isolated from epididymal depots of control and treated rats. Basal glycerol release was not significantly different in adipocytes from treated and control rats (treated 0.87 ± 0.09 μmol of glycerol/100 min/100 mg of total lipids; control 0.94 ± 0.15). Adipocytes from treated rats were supersensitive to isoprenaline and to adrenaline (epinephrine) ($P < 0.05$, Table 1, Figure 1A, C). Treatment with *C. cajucara* extract did not significantly alter the maximal responses to these agonists (Table 1). Neither the sensitivity nor the maximal responses to noradrenaline or BRL37344 were altered in adipocytes from treated rats (Table 1, Figure 1B).

Metoprolol (1 μM) shifted the dose–response curves to isoprenaline to the right in adipocytes from control rats but had no significant effect on adipocytes from treated rats (Table 1, Figure 1A). Metoprolol also shifted the dose–response curve to noradrenaline to the right in adipocytes from control and treated rats but did not significantly modify the maximal responses to the agonist (Table 1, Figure 1B).

ICI118,551 (50 nM) had no effect on the dose–response curve to isoprenaline or noradrenaline in adipocytes from control rats. However, this β_2-adrenoceptor antagonist shifted the dose–response curve to isoprenaline, but not to noradrenaline, to the right in adipocytes from treated rats (Figures 1A, B).

Discussion

Rats receiving *C. cajucara* bark extract for 15 days instead of drinking water showed no significant weight gain whereas control rats showed a significant weight gain during this period. There were no differences in the food or liquid intake between groups. These results show that the extract prevented weight gain, even though food ingestion was not reduced. Because the basal rate of lipolysis was not modified in adipocytes from rats drinking the *C. cajucara* extract, and since there was no change in the weight of epididymal adipose tissue, it seems likely that the treatment somehow altered food absorption or fat metabolism.

Because the adipocyte is the functional unit of adipose tissue, studies on isolated adipocytes may elucidate some of the mechanisms regulating fat metabolism. Our results showed that the sensitivity to isoprenaline and adrenaline was significantly higher in adipocytes from treated rats than from control rats. Since isoprenaline and adrenaline are non-selective β-adrenoceptor agonists, these results suggest an alteration in the adrenoceptor number or affinity to the agonist, or an alteration in some intracellular step following adrenoceptor activation by the agonist induced by treatment of the rats with *C. cajucara* extract.

No changes were observed in the sensitivity or maximum responses to noradrenaline or BRL37344 in adipocytes from rats that received the *C. cajucara* extract. Together, these results indicate that in adipocytes from rats treated with a *C. cajucara* bark extract, there is an increase in the responses mediated by the β_2-adrenoceptor subtype, with no alteration in the β_1- or β_3-adrenoceptor-mediated responses, thus modifying the proportion of β-adrenoceptor subtypes in the adipocytes.

This hypothesis was supported by the effect of antagonists on the responses to agonists. In adipocytes from control rats, metoprolol, a β_1-adrenoceptor selective competitive antagonist, shifted the dose–response curves to isoprenaline and to noradrenaline to the right, with no effect on the maximum responses or basal lipolysis.

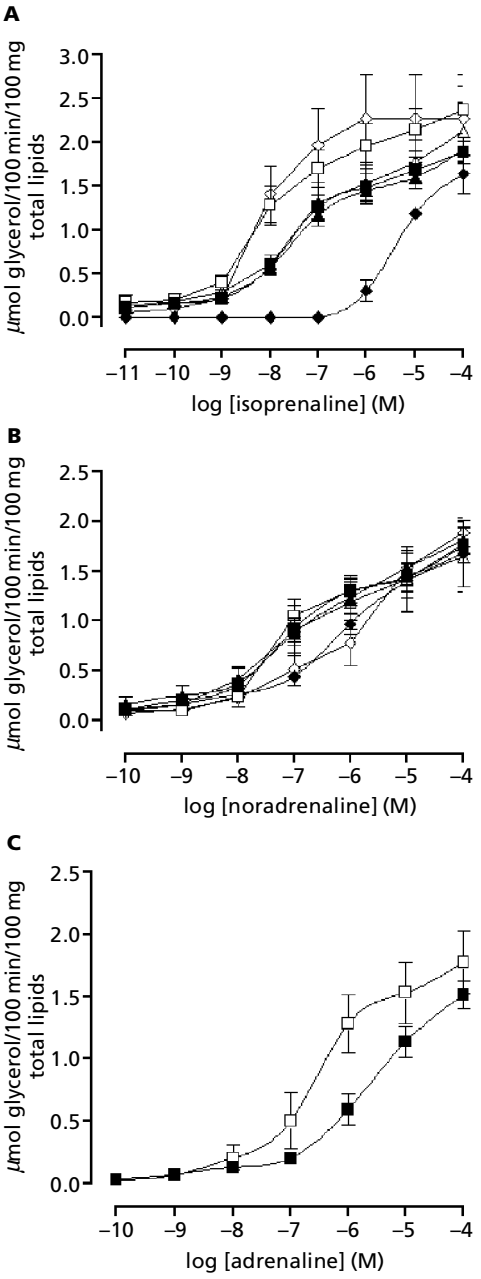

Figure 1 Dose–response curves to isoprenaline (A), noradrenaline (B) and adrenaline (C) in adipocytes isolated from rats treated (full symbols) or not (empty symbols) with *Croton cajucara* Benth in the absence (squares) or presence of antagonists ICI118 (551) (triangles) and metoprolol (diamonds). The points are the means ± s.e.m. of 3–11 experiments performed in duplicate.

However, in adipocytes from treated rats, the dose–response curve to isoprenaline was not modified by metoprolol. This effect agrees with the suggestion that in adipocytes from treated rats the blockade of the β_1-adrenoceptor subtype by metoprolol was compensated for another β-adrenoceptor subtype not blocked by the antagonist but activated by isoprenaline.

Because noradrenaline is selective for the β_1-adrenoceptor subtype, the shift to the right induced by the antagonist in the dose–response curve to noradrenaline was lower than that in the dose–response curve to isoprenaline. Accordingly, the supersensitivity to isoprenaline seen in adipocytes from treated rats was abolished by the selective β_2-adrenoceptor antagonist, ICI118,551, whereas the lipolytic response to noradrenaline was not modified by ICI118,551. In adipocytes from control rats, ICI118,551 had no effect on the dose–response curves to noradrenaline or isoprenaline, which agrees with the fact that in normal rat adipocytes, the β_2-adrenoceptor subtype is of minor importance compared with the β_1- and β_3-adrenoceptor subtypes (Germack et al 1997).

The supersensitivity to non-selective agonists mediated by β_2-adrenoceptors may result from an increase in the number of β_2-adrenoceptors in the adipocyte membrane (Farias-Silva et al 1999), an altered receptor affinity for the agonist or a more efficient coupling between this β-adrenoceptor subtype and the intracellular effectors (Langin & Lafontan 1996). We have not investigated the mechanisms underlying this effect. Regardless of the mechanism, an increase in the β_2-adrenoceptor population would increase the importance of adrenaline as an endogenous stimulator of lipolysis. An increased lipolytic effect of adrenaline could result in increased lipolysis and weight lost, even if food intake were not reduced. Whether this effect occurred in-vivo and its relationship, if any, with the stable body weight of rats during the period they ingested the *C. cajucara* bark extract remain to be demonstrated.

References

Björntorp, P. (1996) The regulation of adipose tissue distribution in humans. *Int. J. Obesity*. **20**: 291–302

Di Stasi, L. C., Hiruma, C. A., Guimarães, E. M., Santos, C. M. (1994) Medicinal plants popularly used in Brazilian Amazon. *Fitoterapia LXV* **6**: 529–549

Dole, V. P., Meinertz, A. (1960) Microdetermination of long chain fatty acids in plasma and tissues. *J. Biol. Chem*. **235**: 2595–2599

Farias-Silva, E., Grassi-Kassisse, D. M., Wolf-Nunes, V., Spadari-Bratfisch, R. (1999) Stress-induced alteration on lipolytic response to β-adrenoceptor agonists in rat white adipocytes. *J. Lipid Res*. **40**: 1719–1727

Germack, R., Starzec, A., Vassy, R., Perret, G. Y. (1997) β-adrenoceptor subtype expression and function in rat white adipocytes. *Br. J. Pharmacol*. **120**: 201–210

Hiruma-Lima, C. A., Gracioso, J. S., Nunes, D. S., Souza Brito, A. R. M. (1999a) Effects of an essential oil from the bark of *Croton cajucara* Benth on experimental gastric ulcer models in rats and mice. *J. Pharm. Pharmacol*. **51**: 341–346

Hiruma-Lima, C. A., Spadari-Bratfisch, R. C., Grassi-Kassisse, D. M., Souza-Brito, A. R. M. (1999b) Antiulcerogenic mechanisms of dehydrocrotonin, a diterpene lactone obtained from *Croton cajucara* Benth. *Planta Med*. **65**: 325–330

Hiruma-Lima, C. A., Gracioso, J. S., Bighetti, E. J. B., Grassi-Kassisse, D. M., Nunes, D. S., Souza Brito, A. R. M. (2002) Effect of essencial oil obtained from *Croton cajucara* Benth. on gastric ulcer healing and protective factors of the gastric mucosa. *Phytomedicine* **9**: 523–529

Kopelman, P. G. (1998) Effects of obesity on fat topography: metabolic and endocrine determinants. In: Kopelman, P. G., Stock, M. J. (eds) *Clinical obesity*. 1st edn, Blackwell Science, Oxford, UK, pp 158–175

Lafontan, M., Berlan, M. (1993) Fat cell adrenergic receptors and the control of white and brown fat cells function. *J. Lipid Res.* **34**: 1057–1091

Lafontan, M., Berlan, M. (1995) Fat cell α_2-adrenoceptors: the regulation of fat cell function and lipolysis. *Endocr Rev.* **16**: 716–738

Langin, C., Lafontan, M. (1996) Adipocyte hormone-sensitive lipase: a major regulator of lipid metabolism. *Proc. Nutr. Soc.* **55**: 93–103

Maciel, M. A. M., Pinto, A. C., Brabo, S. N., Silva, M. N. (1998) Terpenoids from *C. cajucara*. *Phytochemistry* **49**: 823–828

Maciel, M. A. M., Pinto, A. C., Arruda, A. C., Pamplona, S. G. S. R., Vanderlinde, F. A. (2000) Ethnopharmacology, phytochemistry and pharmacology: a successful combination in the study of *Croton cajucara*. *J. Ethnopharm.* **70**: 41–55

Olfert, E. D., Cross, B. M., McWilliam, A. A. (1993) *Guide to the care and use of experimental animals*. Canadian Council on Animal Care 213, Ottawa, Ontario

Rodbell, M. (1964) Metabolism of isolated fat cells. I. Effects of hormones on glucose metabolism and lipolysis. *J. Biol. Chem.* **239**: 375–380

Souza Brito, A. R. M., Nunes, D. S. (1997) Ethnopharmacology and sustainable development of new plant-derived drugs. *Ciênc e Cult.* **49**: 402–408

Souza Brito, A. R. M., Rodríguez, J. A., Hiruma-Lima, C. A., Haun, M., Nunes, D. S. (1998) Antiulcerogenic activity of trans-dehydrocrotonin from *Croton cajucara*. *Planta Med.* **64**: 126–129

Weiland, O. (1957) Eine enzymatische Methode zur bestimmung von glycerin. *Biochem. Z.* **239**: 313–316