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Effects of an N^6-disubstituted adenosine derivative (BM 11.189) on fat cell metabolism

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The action of a number of adenosine derivatives on fat cell metabolism has been studied [1–3]. Of the N^6-substituted adenosine derivatives, the N^6-phenylisopropyl derivative (PIA) has been found to be a good inhibitor of cyclic AMP accumulation and lipolysis in adipose tissue [2] and isolated fat cells [3, 4].

We decided to test the effects of a new disubstituted adenosine derivative, N^6-phenyl-N^6-allyladenosine, designated BM 11.189, on fat cell metabolism. This drug and PIA were gifts of Dr. H. Stork, Boehringer Mannheim Co., Mannheim, West Germany; other chemicals and hormones were from the Sigma Chemical Co., St. Louis, MO.

Fat cells were isolated from the pooled epididymal fat pads of 160–200 g male rats by the procedure of Rodbell [5]. Krebs–Ringer phosphate buffer of the following composition was used in all the experiments: NaCl, 128 mM; $CaCl_2$, 1.4 mM; $MgSO_4$, 1.4 mM; KCl, 5.2 mM; and Na_2HPO_4, 10 mM. The buffer was prepared daily and adjusted to pH 7.4 with NaOH after the addition of Armour fraction V bovine albumin powder (lot 54201). All incubations were done in duplicate for each experiment at 37° in a shaking water bath, in the absence of glucose, with a final volume of 1 ml. The triglyceride content of fat cells was determined as described previously [6]. Samples were removed at the end of the experiments for glycerol analysis [7], and samples were also taken for the determination of free fatty acids [6]. Total cyclic AMP (cells plus medium) was determined by a method published previously [8]. There was no difference in the effect of either PIA or BM 11.189 on intracellular cyclic AMP levels as compared to total cyclic AMP; therefore, the latter data are reported. The cyclic AMP assay itself is a modification of the procedure of Gilman [9], using rabbit muscle protein kinase.

Lipolysis due to 1.5 μM norepinephrine plus 100 μM theophylline was inhibited by both PIA and BM 11.189 at a concentration of 0.1 μM. Figure 1 shows that there was a significant reduction in free fatty acid release due to BM 11.189 and PIA, and Fig. 2 shows a similar result when glycerol release was used as the index of lipolysis. Free fatty acid release can be used as an index of lipolysis in these experiments because of the absence of glucose. In experiments

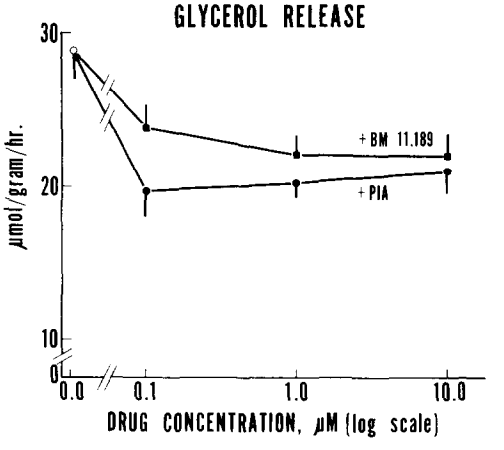

Fig. 2. Effect of PIA and BM 11.189 on glycerol release. Fat cells (28 mg/tube) were incubated for 1 hr in the presence of 1.5 μM norepinephrine plus 100 μM theophylline. PIA and BM 11.189 were present at the concentration indicated. Each value is the mean ± S.E.M. of three experiments done in duplicate.

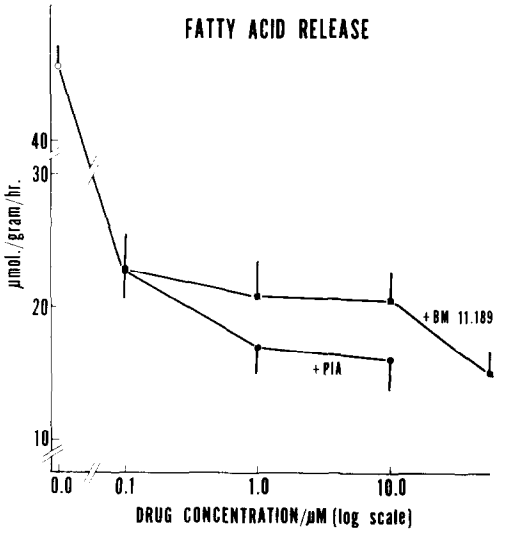

Fig. 1. Effect of PIA and BM 11.189 on fatty acid release. Fat cells (28 mg/tube) were incubated for 1 hr at 37° in the presence of 1.5 μM norepinephrine plus 100 μM theophylline. PIA and BM 11.189 were present at the concentrations indicated. Each value is the mean ± S.E.M. of three experiments done in duplicate.

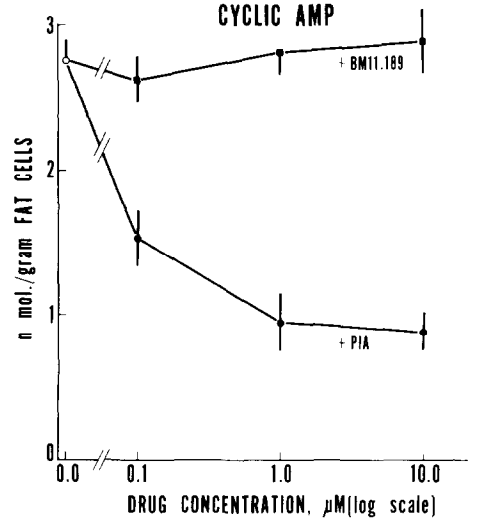

Fig. 3. Effect of PIA and BM 11.189 on cyclic AMP accumulation. Fat cells (34 mg/tube) were incubated for 10 min in the presence of 1.5 μM norepinephrine plus 100 μM theophylline. PIA and BM 11.189 were present at the concentrations indicated. Each value is the mean ±S.E.M. of four experiments done in duplicate.

performed in the presence of glucose, PIA and BM 11. 189 were somewhat less effective in inhibiting fatty acid release and both agents caused a slight potentiation of labeled glucose conversion to triglyceride glycerol, indicating that there was some re-esterification of fatty acid under these conditions (data not shown). Neither drug had any effect on basal lipolysis (data not shown). Figure 3 shows that PIA was able to reduce cyclic AMP accumulation in the presence of norepinephrine (1.5 μM) plus theophylline (100 μM) after a 10 min incubation; however, BM 11. 189 had no effect on cyclic AMP levels in the same series of experiments. PIA has been reported to act in a way similar to that of adenosine which exerts its anti-lipolytic effects by direct inhibition of fat cell adenylate cyclase [10]. BM 11. 189 is a somewhat unique substance in that it is able to inhibit lipolysis, due to norepinephrine plus theophylline, without reducing cyclic AMP levels. Local anesthetics also have been reported to inhibit lipolysis due to high doses of catecholamines without decreasing, or in some cases actually increasing, cyclic AMP levels [11, 12]. However, dibucaine has been shown to inhibit glucose uptake by fat cells [11] whereas BM 11.189 had no effect on basal glucose oxidation in the presence of 0.5 mM glucose, where glucose uptake would be rate limiting, and gave a slight stimulation of glucose oxidation in the presence of 200 μU insulin (data not shown) which was similar to PIA [8]. Kissebah *et al.* [12] have reported that procaine was able to inhibit protein kinase activity in adipose tissue at a dose that also maximally inhibited lipoysis. In preliminary experiments (P. B. Wieser and N. A. Palmeri, unpublished observations), we have found no inhibitory effects of BM 11.189 on protein kinase activity in fat cells. Therefore, it appears that BM 11.189 does not share a similar mechanism of action with local anesthetics and further work will be necessary to determine the precise mechanism of action of BM 11.189 on fat cell metabolism.

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