HYPOGLYCEMIC ACTION OF 2-AMINONORBORNANE-2-CARBOXYLIC ACID IN THE RAT*

H. S. Tager and H. N. Christensen

Department of Biological Chemistry, The University of Michigan
Ann Arbor, Michigan 48104

Received May 19, 1971

SUMMARY: Correct orientation of the amino and carboxyl groups and of the bicyclic ring of the compound 2-aminonorbornane-2-carboxylic acid is essential for its induction of a tolbutamide-potentiated hypoglycemia in the rat, an effect which we have already shown to arise from stimulation of insulin release. Only that isomer with its carboxyl group *exo* and the absolute configuration 1*R*, 2*S*, 4*S* has this action. This finding provides a partial description of a recognition site whereby neutral amino acids stimulate insulin release.

A partially purified preparation of the non-metabolizable amino acid 2-aminonorbornane-2-carboxylic acid (BCH)† produces a tolbutamide-potentiated hypoglycemia in the rat which has been shown to be associated with increases in the immunologically-reactive insulin of the plasma[3]. Using a preparation of BCH enriched in one of the geometric isomers which we call (±)*b*[4], Fajans *et al.*[5] showed that the insulin-releasing action of this compound in the dog resembles that of leucine and differs from that of arginine and lysine in four significant aspects. BCH proved also to be a valuable model substrate for transport, reacting with principal Na^+-independent systems in several animal cells and tissues[4,6], and with a system for branched-chain amino acids in *E. coli*[4]. We have now isolated the four isomers of BCH (Table I) and are reporting here the stereospecificity of their hypoglycemic action in the rat.

---

*Supported in part by grant HD-01233 from National Institutes for Child Health and Human Development, National Institutes of Health, U.S.P.H.S. H.S.T. received support from N.I.H. Training Grant 02919. Preliminary reports of these studies have appeared[1,2].

†The abbreviation used is BCH, 2-aminonorbornane-2-carboxylic acid.

EXPERIMENTAL

The geometric isomers of BCH were prepared by ion-exchange chromatography of the mixtures synthesized by either the Bucherer or the Strecker procedure[4,7]. The racemic pairs, called *a* and *b* (or for greater precision, (±)*a* and (±)*b*), were then resolved as the brucine salts of the N-formyl amino acids[8]. The absolute configurations of the four isomers have been identified by physical, chemical and biochemical techniques[8,9].

The Sprague-Dawley rats used were 50 to 100 g males which were first treated with 20 mmoles/kg of tolbutamide injected intraperitoneally every 12 hr for 48 hr (5 doses). The animals were fasted 20 hr, and the amino acid then injected 2 hr after the last dose of tolbutamide. A sample of fasting blood was collected from the tail, and the test amino acid injected intraperitoneally in a solution containing enough NaCl to make it isoösmolar with blood plasma. Further blood samples were taken at the intervals shown in the figures, and their content of glucose was measured using an ultramicro adaptation of the glucose oxidase assay[10] with the enzyme preparation *Glucostat* of the Worthington Biochemical Co. The decline in the levels for a given interval was calculated by reference to corresponding results with two control rats simultaneously receiving only NaCl solution.

RESULTS

Figure 1 compares the hypoglycemic activity of the two racemic mixtures (±)*a* and (±)*b*, and indicates that only the BCH preparation with the carboxyl group *exo* (the *b* form) is effective in lowering the blood glucose in tolbutamide-primed animals. Although the hypoglycemic action of (±)-b-BCH appears to be both more prolonged and more severe than that of L-leucine, in the dog in contrast the hyperinsulinemic action of BCH is neither as prolonged nor as severe as that of L-leucine[5].

Since the precursor was racemic norbornanone, each preparation of the geometric isomers (*a* and *b*) must necessarily be composed of two optical iso-

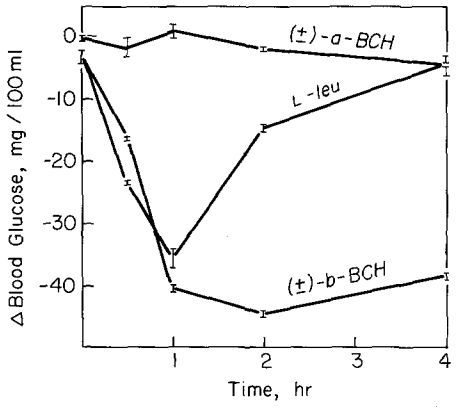
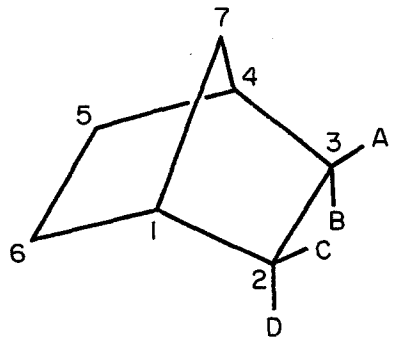


Fig. 1. *Hypoglycemic action of the geometric isomers of BCH.* The racemic mixtures were injected intraperitoneally at a dose of 8 mmoles/kg into fasted, tolbutamide-primed rats as described. The ordinate records the change in blood glucose levels by reference to saline-injected controls. The vertical bars represent duplicate experimental animals. The effect of leucine is shown for reference.

Table I. *Assignment of structures for the four isomers of BCH.* The assignments are summarized from reference 8.



Isomer designation	Substituent at position A	B	C	D
(-)-a-BCH	H	H	NH_3^+	CO_2^-
(+)-a-BCH	NH_3^+	CO_2^-	H	H
(-)-b-BCH	H	H	CO_2^-	NH_3^+
(+)-b-BCH	CO_2^-	NH_3^+	H	H

mers, as shown in Table I. Fig. 2 demonstrates that of the optical isomers present in (±)*b*, only the levorotatory form has measurable hypoglycemic activity. Therefore the dose of biologically active BCH used in the studies of Fig. 1 was really only half that recorded in the legend. Fig. 3 measures the maximally effective dose of (±)-b-BCH under the conditions used, and shows that, at low levels, the effective dose of (-)-b-BCH on a molar basis is half that of the racemic mixture, indicating that no inhibition is exerted by (+)-b-BCH.

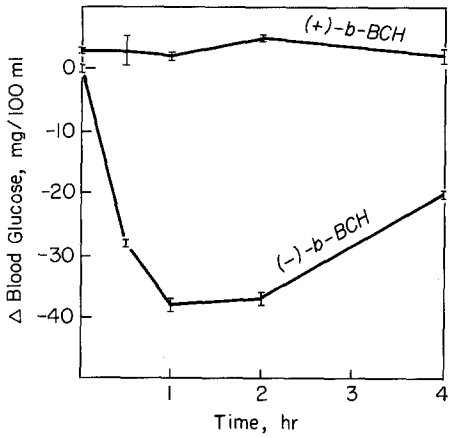


Fig. 2. *Hypoglycemic action of the optical isomers of b-BCH.* The dose was 8 mmoles/kg, the animals being treated as in Fig. 1.

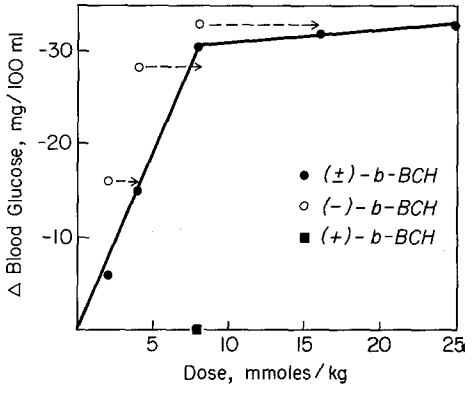


Fig. 3. *Dose-effect relations for (±)-b-BCH and (-)-b-BCH in causing hypoglycemia.* The animals were handled as in Fig. 1. The points plotted are for results 1 hr after administration of the amino acids. The dashed arrows show, when we administered purified (-)-b-BCH, how much (±)-b-BCH would have supplied the same amount of the effective isomer.

Of several other analogs of BCH and leucine tested for their potential hypoglycemic activity, L-isoleucine decreased the blood glucose concentration only about half as much as did L-leucine, whereas the amino acids 1-amino-cyclopentanecarboxylic acid, its 3-methyl derivative, the racemic ω-cyclopentane derivatives of glycine, alanine and α-amino-*n*-butyric acid, the racemic α-methyl-ω-cyclopentane derivatives of glycine and alanine, also L-valine, L-norleucine and D-leucine were ineffective under the conditions used.

2-Methyl-1-aminocyclopentanecarboxylic acid decreased the blood glucose level to a very small extent. In a single experiment by S. S. Fajans and S. Pek, intravenous infusion during 15 min of 16 mmoles of (±)-b-BCH into a 4-kg female dog failed to cause a significant increase in the plasma insulin within an interval of 2 hr.

DISCUSSION

Because of the above differences in the behavior of the isomers of BCH, we were pleased with the proposal of Professor Bo Hellman and his associates at the University of Umeå that the action of this amino acid be studied in microdissected pancreatic islets from an obese, diabetic strain of mice. Again for this preparation, only the levorotatory form of the *b* isomer proved effective in stimulating insulin secretion, although transport studies showed that both (-)-b-BCH and (+)-b-BCH were concentrated by this tissue[11]. One of its advantages is that this preparation can be exposed to any concentration we choose of an amino acid. Quite possibly, a partial effectiveness of some of the amino acids tested for hypoglycemic or insulin-releasing[4,5] action may have been obscured by the difficulty of bringing them to high enough levels in the living animal.

In all other tissues studied in isolation, including segments of hamster intestine, the Ehrlich ascites tumor cell, the pigeon erythrocyte and *E. coli* K-12, we find that the (-)*b* isomer is transported more rapidly than its enantiomorph, and where tested, with greater apparent affinity. The distributions of the enantiomorphs in the intact rat proved, however, rather similar[12].

Reactivity of neutral amino acids with a recognition site for stimulating the secretion of insulin is apparently conditioned by the following structural features: (1) The sidechain should have considerable apolar bulk, which can be provided by branching or ring formation; (2) this bulk should be distributed to correspond to an amino acid in its L configuration; and

(3) a branch on the alpha carbon with the consequent absence of an alpha hydrogen is not detrimental if other steric considerations are met. Beyond these features, we may see that certain dispositions of the mass of the bicycloheptyl ring are unfavorable, and one disposition is favorable. These dispositions can be to some degree mimicked by the isomeric leucines.

We have pointed out earlier how all of the carbon atoms of leucine or isoleucine might be identified each with a carbon atom of BCH[4]. In the case of L-isoleucine the present results do not disturb whatever validity that placement has in describing a possible orientation for this amino acid at the receptor site. If we picture its carboxyl group at position *C* in the sketch of Table I, and its amino group at *D*, then carbon atoms 1, 6 and 5 (or 1, 7 and 4) could represent the principal chain, in which case atoms 7 or 6 would represent the branching methyl group. Carbons 3 and 4 of the sketch will be left over to strut the orientation taken in BCH. In the case of D-leucine we can also identify each of its sidechain carbon atoms with one of the numbered atoms in the sketch, atoms 1 and 6 of the sketch being in that case left over. But since D-leucine appears inert the site apparently does not stabilize the amino acid sufficiently in that or another suitable orientation to produce tight enough binding at the concentrations used. Beginning with the same placement of the carboxyl and amino groups of the effective L isomer of leucine, we may identify only 3 of the 4 atoms of its sidechain with atoms in the sketch, *i.e.*, with atoms 1, 7 and 4 or atoms 1, 6 and 5. The second methyl group of leucine is then left to project from atoms 6 or 7.

We cannot yet say what latitude is acceptable in the placement of a single sidechain carbon atom at the receptor site, because each of these has not yet been varied independently in a known way. All we say is that the bulk represented by the bicyclic ring structure cannot be received effectively if it is shifted a few Ångstroms toward the observer, taking the carboxyl and amino groups as points of reference, nor can it be received effectively if the cyclopentane ring 1,6,5,4,7 is turned upside down.

The structural requirements for the stimulation of insulin release are remarkably similar to those for transport of the above test amino acids by *E. coli*[1,4,12]. In neither case, however, do we propose that the effect arises from a simple, static occupation of the recognition site; that site presumably must first respond with a significant structural change. Description of that change calls for identification of competitive inhibitors that cannot themselves provoke the response. Further complementary description of the biological recognition sites and the factors necessary to their responses should be accessible through the test of additional model substrates

REFERENCES

1. H. S. Tager, H. N. Christensen, A. M. Cullen, S. S. Fajans, H. N. Floyd and R. Zand, *Federation Proc.*, 28, 574 (1969).

2. H. S. Tager, A. M. Cullen and H. N. Christensen, *Federation Proc.*, 29, 300 (1970).

3. H. N. Christensen and A. M. Cullen, *J. Biol. Chem.*, 244, 1521 (1969).

4. H. N. Christensen, M. E. Handlogten, I. Lam, H. S. Tager and R. Zand, *J. Biol. Chem.*, 244, 1510 (1969).

5. S. S. Fajans, R. Quibrera, S. Pek, J. C. Floyd, Jr., H. N. Christensen and J. W. Conn, *J. Clin. Endocr. Metab.*, in press for July 1971.

6. H. S. Tager, T.-C. Shao and H. N. Christensen, *Federation Proc.*, 30, 235 (1971).

7. H. S. Tager and R. Zand, *Anal. Biochem.*, 34, 138 (1970).

8. H. S. Tager and H. N. Christensen, manuscript in preparation.

9. P. Apgar and M. Ludwig, manuscript in preparation.

10. A. St. G. Huggett and D. A. Nixon, *Lancet*, 2, 368 (1957).

11. H. N. Christensen, B. Hellman, A. Lernmark, J. Sehlin, H. S. Tager and I.-B. Täljedal, *Biochim. Biophys. Acta*, in press.

12. H. S. Tager and H. N. Christensen, manuscript in preparation.