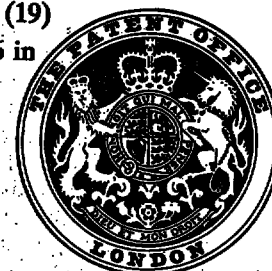


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(54) TRH SOLUTION

(71) We, TAKEDA YAKUHHIN KOGYO KABUSHIKI KAISHA also known as TAKEDA CHEMICAL INDUSTRIES LTD., a body corporate organised under the laws of Japan of 27 Doshomachi 2-chome, Higashi-ku, Osaka, Japan, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:-

The present invention relates to an injectable solution containing L-pyroglutamyl-L-histidyl-L-prolinamide (thyrotropin-releasing hormone; hereinafter referred to briefly as TRH), or a physiologically acceptable salt thereof.

Injectable solutions of TRH or its salts are generally used as a diagnostic reagent for testing the thyrotropin-secreting function of the pituitary gland, and are also of value as drugs for the management of the impaired consciousness due to functional or organic damage of the brain (see German Patent Application (As-Laid-Open) (OLS) No. 26 11 976).

However, the known injectable solution which is prepared merely by dissolving TRH in physiological saline, when administered subcutaneously or intramuscularly, produces an intense pain even if it contains TRH only at a low concentration of the order of 0.1 mg/ml. The intensity of this pain is not substantially altered even if the hydrogen ion concentration of the solution is varied with its osmotic pressure being kept isotonic with sodium chloride, that is to say, even if the pH of the solution is varied within the range of 3 to 7.5 with hydrochloric acid or sodium hydroxide, which phenomenon indicates that the pain is caused by the inherent characteristics of TRH. We have sought to develop an injectable TRH solution rendered substantially painless with a substance which is physiologically completely harmless and is not derogatory to the shelf-life of the solution.

We have found that the concomitant presence of a trihydric to hexahydric sugar alcohol together with TRH or a salt thereof results in a significant mitigation of pain caused on injection.

The present invention has been developed on the basis of that finding.

A major object of the present invention is to provide an injectable solution containing TRH or its salt and a trihydric to hexahydric sugar alcohol with a remarkably reduced pain when administered to a host especially subcutaneously or intramuscularly. Another object is to provide a method of preparing the said injectable solution. Other objects will be made clear from the description and claims hereinafter.

According to the present invention, TRH may be used as the free base or in the form of a physiologically acceptable salt such as an acid addition salt, e.g. an organic acid salt (e.g. acetate, tartrate or citrate) or a mineral acid salt (e.g. hydrochloride or nitrate). The injectable solution of the present invention contains TRH or its salt, in practice in the range of from 0.01 to 5 percent (hereinafter, all percentages are on a weight/volume basis) and, advantageously, in the range of from 0.02 to 2 percent, in terms of free TRH.

The trihydric to hexahydric sugar alcohol of the present invention includes straight-chain sugar alcohols and cyclic sugar alcohols. The straight-chain sugar alcohols may be shown by the general formula $\text{HOCH}_2(\text{CHOH})_n\text{CH}_2\text{OH}$ wherein n is an integer of from 1 to 4, and includes hexitols such as sorbitol, mannitol, allitol, talitol, glucitol, iditol and dulcitol; pentitols such as xylitol, adonitol (also called ribitol) and arabitol; tetritols such as erythritol and threitol; and triols such as glycerol. Among these straight-chain sugar alcohols, hexitols and pentitols, especially hexitols, are advantageously employed. As the cyclic sugar alcohol, there is preferably employed inositol, which may be shown by the formula $\text{C}_6\text{H}_6(\text{OH})_6$ and

exemplified by meso-inositol. While some of those sugar alcohols exist in stereoisomers (geometric isomers or/and optical isomers), the present invention can be practised with the employment of any of such forms of alcohols. These sugar alcohols may be employed alone or in various combinations.

To accomplish the object of making an injection painless, the sugar alcohol is desirably added at a level that will make the osmotic pressure of the injectable solution substantially isotonic, with body fluids, especially just isotonic or slightly hypertonic. Although the level of addition of the sugar alcohol varies with the concentration of TRH or its salt and the particular sugar alcohol used, it is generally advantageous that the alcohol should be incorporated in the solution at a level of from 2 to 9 percent and, for still better results, from 4 to 8 percent.

The preparation of the injectable solution of the present invention can be accomplished by a procedure similar to that known in the art. For example, an injectable solution can be produced by dissolving TRH or a salt thereof and the sugar alcohol in distilled water for injection and adjusting the pH of the mixed solution. The pH of the solution is normally adjusted to the range of from 3 to 7.5; preferably the range of from 3.5 to 6.5, especially around 6. For this adjustment of pH, there may conveniently be employed, for example, hydrochloric acid or sodium hydroxide which may be chosen depending upon the form of TRH employed, i.e. the free base of the physiologically acceptable salts.

In practice, it is advantageous to make the injectable solution substantially free from other electrolytes, as solutes, than TRH or its salt and the sugar alcohol. For example, the injectable solution desirably does not contain any substantial amount (e.g. not more than 0.1%) of sodium chloride, because it will interfere with the pain-reducing action of the sugar alcohol. Thus, it is most advantageous that the injectable solution should consist essentially of TRH or its salt and the sugar alcohol as solutes and water as solvent and the pH thereof is preferably adjusted to the range of from 3 to 7.5. It is to be noted that the said pH adjustment can be achieved by the addition of a small amount (e.g. less than 0.1%) of hydrochloric acid or sodium hydroxide.

By virtue of the concomitant presence of the sugar alcohol, the injectable solution of this invention is remarkably reduced in its pain producing effect and is stable as compared with the conventional TRH injections, with no particular side-effects being noticed. Therefore, it is suitable, especially, for subcutaneous or intramuscular administration to a host (human beings; domesticated animals, e.g. dogs and cats; laboratory animals, e.g. rats and mice).

The injectable solution of the present invention may be employed in the same manner as the conventional TRH injectable solutions as, for example, a diagnostic reagent for testing the thyrotropin-secreting function and as a drug for the treatment of the impaired consciousness.

The effect of the present invention will hereinafter be described by way of experimental data and working examples which, however, should not be construed as limiting the scope of the invention.

Experiment

Aqueous solutions were prepared by incorporating 0.5 mg. (in terms of free TRH) of TRH or TRH tartrate in 1 ml. portions of distilled water for injection, and the solutions were each rendered isotonic by the addition of one of the sugar alcohols mentioned in Table 2 or sodium chloride as a control and adjusted to the pH value mentioned in Table 2 with hydrochloric acid or sodium hydroxide. Each aqueous solution was filtered through a membrane filter and filled into a white ampoule of 1 ml. capacity, which was sealed and autoclaved. The injectable solutions thus obtained were subjected to a pain test using rabbits. The testing procedure comprised injecting 0.2 ml of each test product subcutaneously into the dorsal ear of rabbits that were under unrestricted conditions. The intensity of pain was scored on the scale of Table 1 based on the degree of escaping motion and the behaviour of the rabbits at and after the injection.

(12)

Table 1

Score	Observation at and after injection	
5	- No pain reaction	5
	+ A slight escaping reaction at injection; the animals shake their head a few times after injection	
10	+ + A fairly prominent escaping reaction at injection; the animals shake their heads violently after injection	10
	+ + + A strong escaping reaction; the animals cry	
15		15

The results, as shown in Table 2, clearly indicate that, while sodium chloride-isotonicated aqueous solutions of TRH or its salt produced intense pains, the sugar alcohol-isotonicated injectable solutions of this invention invariably did not elicit pain reactions.

Table 2

20	Additive and its concentration (per ml.)	Form of TRH	pH	Degree of pain	20
25	Sodium chloride 9 mg.	Free	3.5	++	25
	Sodium chloride	Free	4.5	++	
	Sodium chloride	Free	5.5	++	
	Sodium chloride	Free	6.5	++	
	Sodium chloride	Tartrate	3.5	+++	
30	Sodium chloride	Tartrate	4.6	++	30
	Sodium chloride	Tartrate	5.5	++	
	Sodium chloride	Tartrate	6.6	++	
35	D-mannitol 50 mg.	Free	4.1	-	35
	D-mannitol	Free	6.0	-	
	D-mannitol	Tartrate	3.9	-	
	D-mannitol	Tartrate	6.0	-	
40	meso-Inositol 50 mg.	Free	4.1	-	40
	meso-Inositol	Free	6.0	-	
	meso-Inositol	Tartrate	4.0	-	
	meso-Inositol	Tartrate	5.9	-	
45	D-sorbitol 50 mg.	Free	4.1	-	45
	D-sorbitol	Free	6.1	-	
	D-sorbitol	Tartrate	4.1	-	
	D-sorbitol	Tartrate	5.9	-	
50	D-xylitol 50 mg.	Free	4.0	-	50
	D-xylitol	Free	6.1	-	
	D-xylitol	Tartrate	3.9	-	
	D-xylitol	Tartrate	5.9	-	
55	Glycerol 19.4 mg.	Free	3.9	-	55
	Glycerol	Free	6.0	-	
	Glycerol	Tartrate	4.1	-	
	Glycerol	Tartrate	6.0	-	

Example 1

60	An aqueous solution was prepared by incorporating 0.732 mg. of TRH tartrate and 50 mg. of meso-inositol in 1 ml. of distilled water for injection and adjusting the mixture to pH 4.0 with sodium hydroxide. The solution was filtered through a membrane filter and filled into a white ampoule of 1 ml. capacity, which was then sealed and autoclaved. By this procedure there was obtained an injectable solution.	60
65		65

Example 2

An aqueous solution was prepared by incorporating 0.732 mg. of TRH tartrate and 50 mg. of D-sorbitol in 1 ml. of distilled water for injection and adjusting the mixture to pH 6.0 with sodium hydroxide. The solution was processed as in Example 1 to yield an injectable solution.

Example 3

An aqueous solution was prepared by incorporating 1 mg. of TRH and 19.4 mg. of glycerol in 1 ml. of distilled water for injection. The solution was processed as in Example 1 to yield an injectable solution.

Example 4

The procedure of Example 1 was repeated except that 50 mg. of D-xylitol were used in lieu of meso-inositol to prepare an injectable solution.

Example 5

The procedure of Example 2 was repeated except that 50 mg. of D-mannitol were used in lieu of D-sorbitol to prepare an injectable solution.

WHAT WE CLAIM IS:-

1. An injectable solution, which contains L-pyroglutamyl-L-histidyl-L-prolinamide or its physiologically acceptable salt and a trihydric to hexahydric sugar alcohol as solutes and water as solvent.
2. A solution as claimed in claim 1, wherein the sugar alcohol is a straight-chain alcohol.
3. A solution as claimed in claim 2, wherein the straight-chain sugar alcohol is a hexitol or pentitol.
4. A solution as claimed in claim 1, wherein the sugar alcohol is a hexitol.
5. A solution as claimed in claim 4, wherein the hexitol is D-sorbitol.
6. A solution as claimed in claim 1, wherein the sugar alcohol is a cyclic alcohol.
7. A solution as claimed in claim 6, wherein the cyclic sugar alcohol is inositol.
8. A solution as claimed in any of claims 1 to 7, which is substantially isotonic with body fluids.
9. A solution as claimed in claim 1, wherein the concentration of the sugar alcohol is in the range of from 2 to 9 percent and the concentration of L-pyroglutamyl-L-histidyl-L-prolinamide or its physiologically acceptable salt is in the range of from 0.01 to 5 percent in terms of free L-pyroglutamyl-L-histidyl-L-prolinamide.
10. A solution as claimed in claim 1, which is substantially free from sodium chloride.
11. A solution as claimed in claim 1, the pH of which is in the range of from 3 to 7.5.
12. A solution as claimed in claim 1, which consists essentially of L-pyroglutamyl-L-histidyl-L-prolinamide or its physiologically acceptable salt and a trihydric to hexahydric sugar alcohol as solutes and water as solvent, and the pH of which is adjusted to the range of from 3.5 to 7.5.
13. A solution as claimed in claim 1, wherein the physiologically acceptable salt is a tartrate.
14. An injectable solution as claimed in claim 1, substantially as herein described with reference to any of the specific examples.
15. A method of preparing an injectable solution as claimed in any one of claim 1 to 14, which comprises dissolving L-pyroglutamyl-L-histidyl-L-prolinamide or its physiologically acceptable salt and a trihydric to hexahydric sugar alcohol in water.
16. A method as claimed in claim 15, substantially as herein described with reference to any of the specific examples.
17. A mixture of L-pyroglutamyl-L-histidyl-L-prolinamide or its physiologically acceptable salt and a trihydric to hexahydric sugar alcohol, adapted to be dissolved in water to prepare an injectable solution as defined in claim 1.

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*Example 2*

An aqueous solution was prepared by incorporating 0.732 mg. of TRH tartrate and 50 mg. of D-sorbitol in 1 ml. of distilled water for injection and adjusting the mixture to pH 6.0 with sodium hydroxide. The solution was processed as in Example 1 to yield an injectable solution.

Example 3

An aqueous solution was prepared by incorporating 1 mg. of TRH and 19.4 mg. of glycerol in 1 ml. of distilled water for injection. The solution was processed as in Example 1 to yield an injectable solution.

Example 4

The procedure of Example 1 was repeated except that 50 mg. of D-xylitol were used in lieu of meso-inositol to prepare an injectable solution.

Example 5

The procedure of Example 2 was repeated except that 50 mg. of D-mannitol were used in lieu of D-sorbitol to prepare an injectable solution.

WHAT WE CLAIM IS:-

1. An injectable solution, which contains L-pyroglutamyl-L-histidyl-L-prolinamide or its physiologically acceptable salt and a trihydric to hexahydric sugar alcohol as solutes and water as solvent.
2. A solution as claimed in claim 1, wherein the sugar alcohol is a straight-chain alcohol.
3. A solution as claimed in claim 2, wherein the straight-chain sugar alcohol is a hexitol or pentitol.
4. A solution as claimed in claim 1, wherein the sugar alcohol is a hexitol.
5. A solution as claimed in claim 4, wherein the hexitol is D-sorbitol.
6. A solution as claimed in claim 1, wherein the sugar alcohol is a cyclic alcohol.
7. A solution as claimed in claim 6, wherein the cyclic sugar alcohol is inositol.
8. A solution as claimed in any of claims 1 to 7, which is substantially isotonic with body fluids.
9. A solution as claimed in claim 1, wherein the concentration of the sugar alcohol is in the range of from 2 to 9 percent and the concentration of L-pyroglutamyl-L-histidyl-L-prolinamide or its physiologically acceptable salt is in the range of from 0.01 to 5 percent in terms of free L-pyroglutamyl-L-histidyl-L-prolinamide.
10. A solution as claimed in claim 1, which is substantially free from sodium chloride.
11. A solution as claimed in claim 1, the pH of which is in the range of from 3 to 7.5.
12. A solution as claimed in claim 1, which consists essentially of L-pyroglutamyl-L-histidyl-L-prolinamide or its physiologically acceptable salt and a trihydric to hexahydric sugar alcohol as solutes and water as solvent, and the pH of which is adjusted to the range of from 3.5 to 7.5.
13. A solution as claimed in claim 1, wherein the physiologically acceptable salt is a tartrate.
14. An injectable solution as claimed in claim 1, substantially as herein described with reference to any of the specific examples.
15. A method of preparing an injectable solution as claimed in any one of claim 1 to 14, which comprises dissolving L-pyroglutamyl-L-histidyl-L-prolinamide or its physiologically acceptable salt and a trihydric to hexahydric sugar alcohol in water.
16. A method as claimed in claim 15, substantially as herein described with reference to any of the specific examples.
17. A mixture of L-pyroglutamyl-L-histidyl-L-prolinamide or its physiologically acceptable salt and a trihydric to hexahydric sugar alcohol, adapted to be dissolved in water to prepare an injectable solution as defined in claim 1.

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