

Test requested by Litanial Bio Science. Co., Ltd

**TEST RPORT of The LBS Culture effect
through LBS application on the rat skin to evaluate the activity of the artery sympathetic nerve
and
adrenal sympathetic nerve**

Test #: ANBAS 00334

Date: Aug. 7th, 2013

Made by: ANBAS Co., Ltd

Exam Outline

1. Test Title:

Studies of effect by LBS Culture application on the skin to the activity of the artery sympathetic nerve and the activity of rat adrenal sympathetic nerve.

2. Test Purpose:

According to the experiment, LBS culture (LBS extract) suppress the itching of the skin, increase the coercive humidity, improvement of acne, promote wound healing, obtain face skin tightness, and very little issues with skin negative reaction.

It is well known when the adrenal sympathetic nerve is suppressed reduces the pain nerve impulse, the conduction velocity, resulting to reduce the pain and itching sensation. Therefore, when adrenal nerve is suppressed improves itching sensation and when the cutaneous arteries sympathetic nerve is suppressed blood flow increases, and when cutaneous arteries sympathetic nerve is suppressed leads to vascular relaxation for more blood flow, increasing the coercive humidity of the skin, wound healing and hair growth promotion.

Therefore in this LBS culture study, we aimed to clarify the mechanism of skin blood flow increasing effect of action and suppression itching of the skin, to measure the changes in the activity of the skin artery sympathetic nerve and adrenal sympathetic nerve skin after application.

3. Test Requested by:

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6. Tested Period:

Test begun: July 11th 2013

Test ended: August 6th, 2013

7. Test Substance: LBS Culture stock solution, solvent (Medium before fermentation)

1. Introduction:

We have examined the energy metabolism regulation mechanism by the control mechanism of the autonomic nervous system in rats. In that process, we found changes in the environment from inside and/or outside excite sympathetic nerves which dominate adrenal glands, liver, kidney and pancreas and raises the blood pressure and blood sugar; on the other hand, substances that reduce the neural activity of those organs gained opposite results indicate that lowering blood pressure and blood sugar level.

In addition, the sent stimulation of grapefruit essential oil commonly known to excite sympathetic nervous system which dominates white adipose tissue, brown adipose tissue, adrenal glands, and the kidney and suppresses the parasympathetic nerves which dominate the stomach by raising the blood pressure to promote (heat production) and energy consumption lipolysis, to suppress the appetite resulting the weight reduction.

Whereas, the sent stimulation with lavender essential oil is commonly known to suppresses sympathetic nervous system which dominates white adipose tissue, brown adipose tissue, adrenal glands, and promotes the parasympathetic nerves which dominate the stomach by lowering blood pressure to suppress the (heat production) energy consumption and lipolysis and to enhance the appetite.

In addition, Lactic acid bacteria, *Lactobacillus johnsonii* La1 (NCC533) and *Lactobacillus paracasei* ST11 (NCC2461) are respectively known to,

- 1) Promote and inhibit the activity of adrenal sympathetic centrifugal branch,
- 2) Suppress and promote the activities of the (parasympathetic) nerve centrifugal branch stomach vagus,
- 3) Result to suppress and promote appetite.

According to experimental study we've learned that LBS culture (LBS extract) suppress the itching of the skin, increase the coercive humidity, improvement of acne, promote wound healing, obtain face skin tightness, and very little issues with skin negative reaction.

It is well known when the adrenal sympathetic nerve is suppressed reduces the pain nerve impulse, the conduction velocity, resulting to reduce the pain and itching sensation. Therefore, when adrenal nerve is suppressed improves itching sensation and when the cutaneous arteries sympathetic nerve is suppressed blood flow increases, and when cutaneous arteries sympathetic nerve is suppressed leads to vascular relaxation for more blood flow, increasing the coercive humidity of the skin, wound healing and hair growth promotion.

Therefore in this LBS culture study, we aimed to clarify the mechanism of action of increasing skin blood flow and suppression effect itching sensation. Our main objective was to measure the change in activity of the skin and arteries sympathetic nerve adrenal nerve skin after application of LBS culture.

2. Methods

In the experiment, each male Wistar rats of about 300g body weight (about 9 weeks old) were housed for about one week and more in a homeothermy room of 24 °C (lit until 8:00 to 20:00) under light-dark cycle of 12 hours. The subjects were administrated with urethane anesthesia after fasted for 3 hours the day of the experiment, lifting skin artery sympathetic nerve of the left thigh front centrifugation or branch of adrenal sympathetic nerve by a silver electrode measured the electrical activity of the nerve method described above. When this instrument measurement became steady (13 o'clock), the LBS Culture 2ml was applied to the entire tail and right entire thigh (shaved entire thigh circumference) covered by polyvinylidene chloride made packaging wrap (Saran Wrap), and measured changes in neural activity electrophysiologically using three rats. As a comparative experiment, used three rats and measured the changes in two neural activities in a similar manner with the LBS culture 2ml medium applications. In addition, to ensure the airway by inserting a tube into the trachea until the end of measurement from start of surgery, and kept to 35.0 ± 0.5 °C body temperature (rat rectal temperature) in thermal insulation equipment. Sympathetic activity data of these analyzed by average firing rate of 5 per second for 5 minutes each (pulse / 5 s), expressed in percentage with 100% values of 5 minutes before applying stimulation (0 min values). In addition, calculate the mean \pm standard error of data, test of statistical significance as a group was conducted in analysis of variance (ANOVA) with repeated measures, carried out by Mann-Whitney U-test to test the statistical significance of the absolute value between the neural activity of (0 min) applied before stimulus onset.

3. Results

3.1. Study of the Effects of cutaneous application of LBS Culture for adrenal sympathetic nerve activity.

3.1.1. Exploratory study

In order to examine the effective amount of the coating to the skin, we prepared 6 types of solutions as such the LBS culture stock, 10 times, 100 times, 1,000 time, and 10,000 times diluted with medium stock solution and the medium stock solution alone and each are measured 2ml applied to see the change of the skin artery sympathetic nerve activity (cutaneous arterial sympathetic nerve activity, CASNA) of the thigh of urethane anesthetized each rats. FIG 4 shows skin actual measurement change data in CASNA at this time indicating the skin stimulation before application of the CASNA neural activity (0 min) as 100%.

As a comparative study, the medium solution 2ml dermal application changed CASNA value just a little and raised to 104.7% maximum after 60 minutes (FIG 4). On the other hand, skin irritation with LBS culture 2ml 10,000-fold dilution of stock solution dermal application shows CASNA value 126.3% maximum after 15 minutes, the other measurement period the middle of CASNA value remain in the middle (Figure 4). The application of LBS culture 2ml of 1,000-fold dilution of the stock solution shows little change in CASNA value, after 25 minutes 107.8% maximum and 90.2% minimum after 60 minutes and other measurement period indicated in the middle of CASNA value (Figure 4). The application of LBS culture 2ml of 100-fold dilution of the stock solution shows gradual decline in CASNA value, after 60 minutes down to 74.4% minimum and other measurement period indicated in the middle of CASNA value (Figure 4). The application of LBS culture 2ml of 10-fold dilution of the stock solution shows early decline

in CASNA value down to 79.2% minimum after 10 minutes and gradually increased to 100.008% maximum after 55 minutes (Figure 4). On the other hand, skin irritation with LBS culture stock solution 2ml dermal application increased CASNA value slightly

Increased to 115.4% maximum to 15 minutes after dermal application of stimulus onset the CASNA value, then, is reduced slightly to 97.8% minimum to 35 minutes after dermal application of stimulus onset, the value of their ASNA value of the measurement period in the other was stopped on the value of the intermediate (Figure 4).

3.1.2. Confirmatory study

FIG 2 shows the measured data of ASNA when LBS culture is applied to the skin with the medium solution 2ml or 100-fold dilution of stock solution, FIG 3 shows in percentage with 100% of the neural activity of ASNA (0 min) before applied on the skin irritation. As a comparative study, a medium solution 2ml applied to skin irritation shows just little changing in ASNA, and then ASNA value shows $101.6 \pm 6.5\%$ maximum after 20 minutes applied the stimulation, and then after 40 minutes application stimulus onset, ASNA shown $93.8 \pm 6.2\%$ minimum, then other period of the measurement shows in the middle of the ASNA value (Figure 3). On the other hand, the application of LBS culture 100-fold dilution stock solution 2ml go gradually decreased ASNA skin irritation, then the ASNA value was reduced to $72.9 \pm 0.9\%$ minimum after 55 minutes the start of the stimulation applied on the skin (Figure 3). When you consider statistical ASNA value difference after 5 to 60 minutes between the LBS culture medium solution and the 100-fold dilution, the diluted solution group is significantly lower ASNA value ($P < 0.0005$, $F = 59.5$ by ANOVA with repeated measures) than the medium solution application group. Table 1 shows the absolute value of ASNA in (0 min), before 100-fold diluted LBS culture stimulation and the medium solution applied groups, between the absolute value of the ASNA (0 min) in these two groups, there was no statistically significant difference by Mann-Whitney U-test.

3.2. Study of the skin artery sympathetic nerve activity by LBS Culture cutaneous application.

3.2.1. Exploratory study.

Firstly, in order to find out the amount of the application effect to the skin, 6 types solutions are arranged such the LBS culture stock solution, diluted with medium solution to 10 times, 100 times, 1000 times, 10000 times and medium solution. Those each 2ml was applied to the thigh skin to the urethane anesthetized rats in order to measure skin sympathetic nerve artery activity (cutaneous arterial sympathetic nerve activity, CASNA). Fig 4 shows in 100% percentage of the neural activity of CASNA (0 min) before measurement applied on the skin. As a comparative experiment, the medium solution 2ml dermal application of the stimulus onset CASNA value raised a little and increased to 104.7% maximum to 60 minutes after. On the other hand, the LBS culture 2ml of 10,000-fold dermal application showed CASNA value 126.3% maximum in 15 minutes and other value remained the middle in between 100% (Figure 4). The 2ml LBS culture 1,000-fold dilution dermal application showed little variation in CASNA value, after 25 minutes 107.8% maximum, after 60 minutes 90.2% minimum and other value remained in the middle of these values (Figure 4). The 2ml LBS culture 100-fold dilution dermal application showed gradual decrease in CASNA value to minimum 74.0% after 60 minutes (Figure 4). The 2ml LBS culture 10-fold dilution dermal application decreased CASNA value to 79.2% minimum to 10 minutes then gradually increased to 100.008% maximum after 55 minutes. The 2ml LBS culture stock solution dermal application increased CASNA value to 115.4% maximum after 15 minutes, then after slightly decreased

to 97.8% after 35 minutes, other value remained in the middle of these values. Above exploratory study shows the LBS Culture 100-fold dilution of stock solution decrease CASNA value most strongly, we examined confirmatory of CASNA value using three rats.

3.2.2. Confirmatory study

Figure 5 shows the measured date of the CASNA value to the skin when the medium solution 2ml and LBS culture 100-fold dilution of the stock solution, Figure 6 shows in percentage with 100% of the neural activity of CASNA (0 min) before applied on the skin irritation. As a comparative study, skin irritation experiment by coating with 2ml medium solution shown with little change in CASNA value, 96.7+-2.7% minimum after 10 minutes and 105+-2.7% maximum after 55 minutes and remained in the middle of the these values in the rest of the time (Figure 6). On the other hand, skin irritation application of 100-fold of the 2ml LBS Culture stock solution shown gradual decrease to 89.7+-7.9% of CASNA value after 60 minutes (Figure 6). The statistical study of SCNA value between medium solution and 100-fold diluted LBS culture stock solution after 5 minutes to 60 minutes, LBS culture application group shown significantly lower CASNA value ($P<0.0005$, $F=38.0$ by ANOVA with repeated measures) than the contrast medium solution group. Table 2 shows the CASNA absolute value before (0 min) of LBS culture stimulation application and the control medium solution group, and shown no statistically signification difference by Mann-Whitney U-test.

4. Consideration

The above comparative experiments in skin irritation to urethane-anesthetized rats between the medium solution liquid group and LBS Culture 100-fold diluted stock solution group, 1) 2ml LBS culture 100-fold group application to skin irritation decreases significantly adrenal sympathetic nerve activity (ASNA) (Figure 3), 2) 2ml LBS culture 100-fold group application to skin irritation decreases significantly artery skin sympathetic nerve activity (CASNA) (Figure 6). Therefore, the suppression of adrenal sympathetic nerve leads to the reduction of the nerve impulse, resulting the itching and pain is suppressed. In addition, when skin sympathetic artery is suppressed, relaxes vessel for more blood flow to increase the coercive humidity of the skin, promotes wound healing and hair growth. The study results with LBS culture cutaneous application increases blood flow in the skin to enhance the protection of the skin moisture, suggesting the effect of promoting wound healing and hair growth.

5. Conclusion

We investigated the effect of LBS Culture skin application using urethane anesthetized rats to adrenal sympathetic nerve activity (ASNA) and skin sympathetic nerve and artery and found the LBS culture skin application reduces adrenal sympathetic nerve activity resulting the reduction of itching, suppressing the skin artery sympathetic nerve activity to enhance the protection of the skin moisture, promote hair growth, and to promote wound healing.

References

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図1 LBSカルチャーの原液、原液の培地溶液による10倍希釈液、100倍希釈液、1000倍希釈液、10,000倍希釈液および培地溶液の胃内投与による腸迷走神経活動(intestinal vagal nerve activity、intesimal vagal-NA) の変化

Figure 1: LBS Culture Stock Solution, 1/10 Medium solution 1/100 Medium solution, 1/1,000 Medium solution, 1/10,000 Medium solution and Medium solution intragastric application stimulation resulting changes in the intestinal vagus nerve (intestinal vanga nerve activity, intesimal vagal-NA) change.

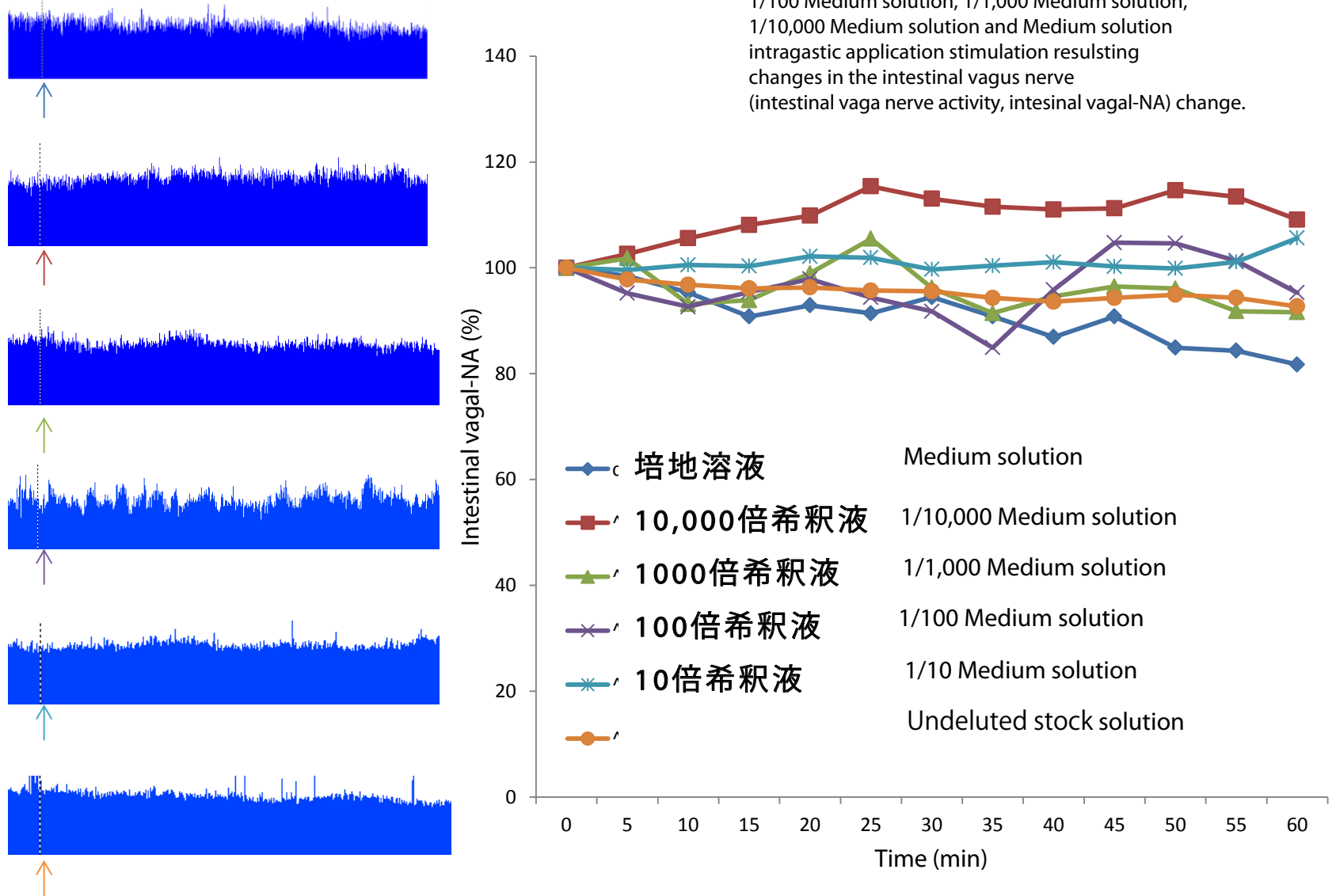


図2 LBSカルチャーの原液の培地溶液による10,000倍希釈液および培地溶液の胃内投与による腸迷走神経活動 (intestinal vagal-NA) の変化 (実測データ)

Figure 2: Comparative study between Medium solution and LBS Culture 1/10,000 Medium solution intragastric application of changes in the intestinal vagus nerve (intestinal vago nerve activity, intestinal vagal-NA)

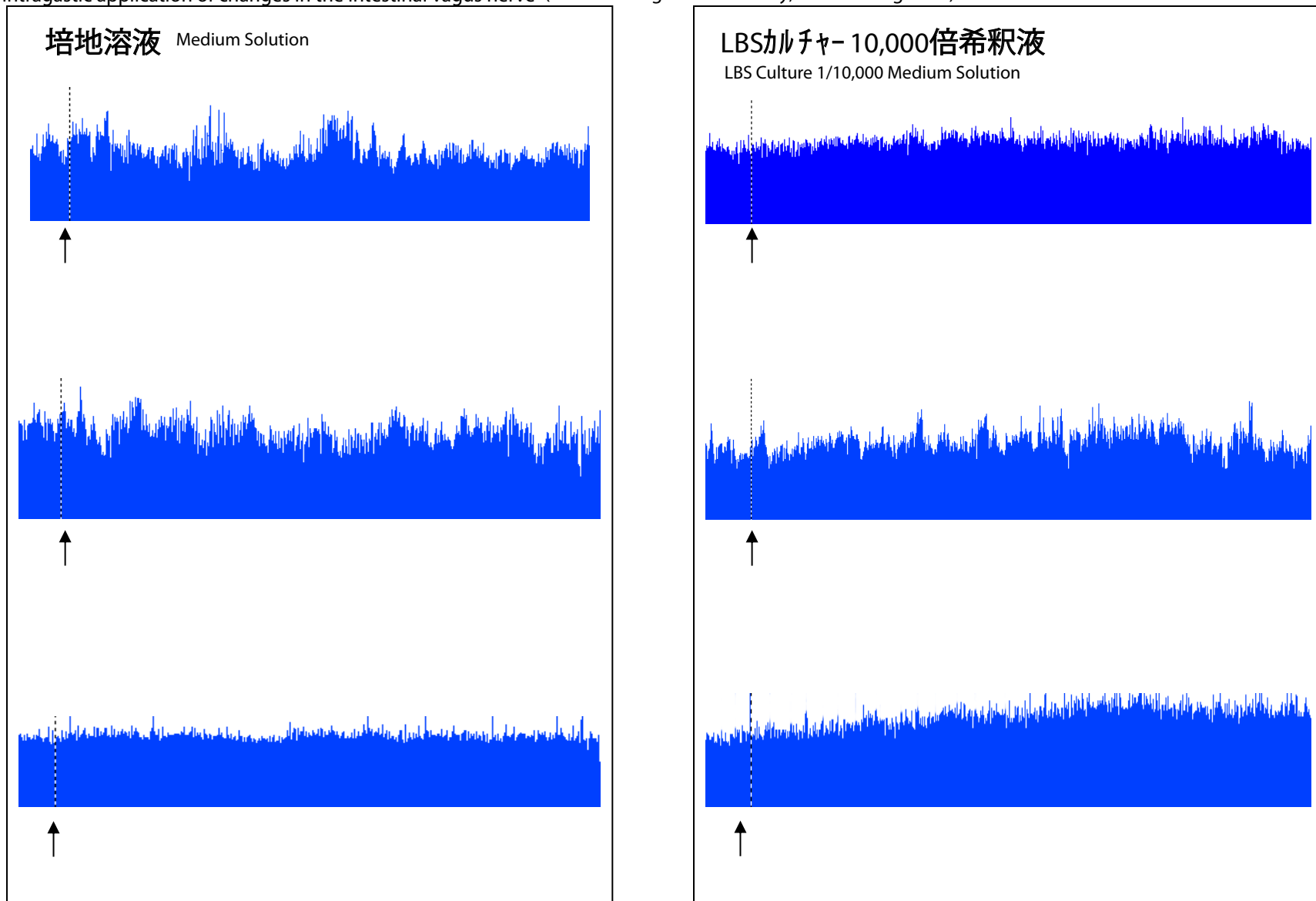


図3 LBS加肝臓の原液の培地溶液による10,000倍希釈液および培地溶液の胃内投与による腸迷走神経活動(intestinal vagal-NA)の変化

Figure 3, LBS Culture medium solution and its 1/10,000 medium solution intragastric application and changes in intestinal vagus nerve (intestinal vagal-NA).

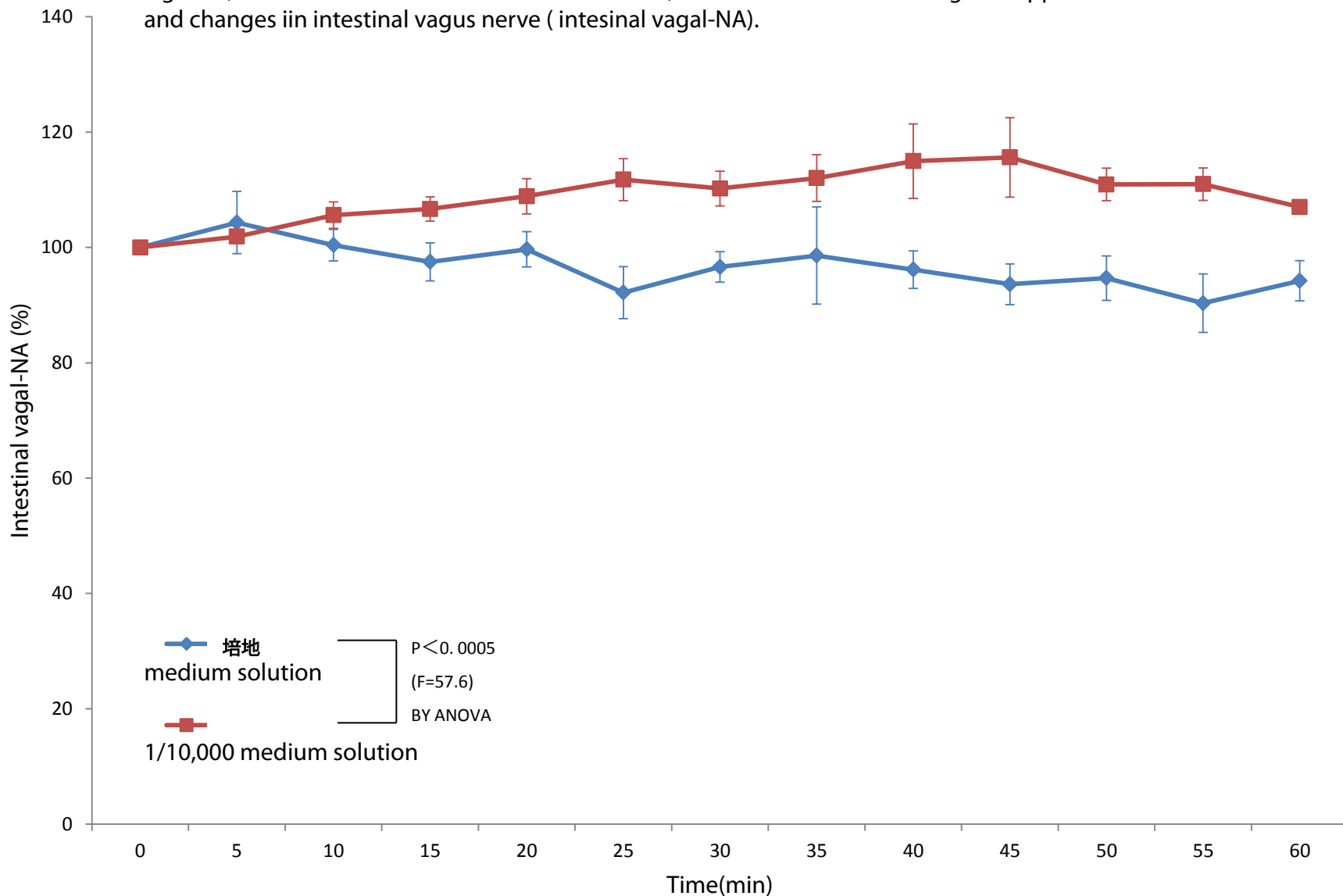


表1 培地溶液もしくはLBSカルチャー 1万倍希釈液の胃内投与直前（0分）の Intestinal vagal-NAの絶対値

Table 1, Intestinal vagal-NA absolute Value before Medium solution and LBS culture 1/10,000 medium solution application (0 min)

実験群 Experimental group	神経活動 (spikes/5 s)(Mean± SEM) Nerve activity
培地溶液 Medium Solution	256± 3
LBSカルチャー LBS Culture 1/10,000 medium solution	242± 24

Mann-Whitney U-testによる統計的有意差なし
No statistically significant difference