CONTRALATERAL EFFECT OF ADJUVANT INDUCED MONOARTHRITIS

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Abstract - Experiments were performed to evaluate the contralateral effect of adjuvant induced mono arthritis. In these experiments Laser Doppler imaging (LDI) was used to investigate changes in blood flow. Adjuvant was applied to induce chronic inflammation.Temperature and knee diameter increased in the first week after administration in the administrated knee joint.

Substance p (SP)(a neurokinin with a vaosodjlatory effect) was applied topically to the adjuvant treated knee joint and adjuvant altered and attenuated the effect of SP on blood flow, which was consistent during the three weeks. In the other knee joint (contralateral) inflammatory effect of the adjuvant was revealed during these experiments. An increase in the temperature and knee diameter and alteration of the effect of SP on blood flow was observed in the contralateral knee joint during week one after administration. The increased diameter and temperature abated in week two and three. As the diameter and temperature of ankle jointes did not change during the experiments in both ankle it suggests a neurogenic contralateral effect of arthritis.

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Key Words: LDI, Adjuvant, substance P, inflammation

INTRODUCTION

Adjuvant was described over 40 years ago (12) as an agent for induction of arthritis. Adjuvant is made from a variety of dead mycobacteria, usually mycobacterium tuberculosis and mycobacterium buturicum or mycobacterium phelei, suspended in vegetable or mineral oil. Corinea bacterium and nocardia asteroides have also been used to induce arthritis (2). The contralateral effect of neurogenic arthritis began to be recognised some years ago. Axotomy of the nerve to one muscle of the frog induced sprouting and synapse formation by the homologous intact nerve on the opposite side and this has since been observed in three different muscles, the cutaneous pectoralis (11), the sartorious (9), and piriformis (4). Similar events also occurred in mammals and it was found that in the intact muscle of the normal rat, spouting and synapse formation is an ongoing process which can be enhanced by contralateral axotomy (10). Bilevicuite (3) showed bilateral changes in synovial fluid neuropeptide content to monoarthriti's.

Allnatt (1) reported that sapheneous nerve injury and degeneration in one rat leg suppressed the ability of the contralateral nerve to evoke plasma extravasation, which indicated that there is a neuronic contralateral effect in the body organs. Kidd (5) reported that synovial drainage resulted in acute inflammation in the damaged joint and in a neurogenically mediated infiltrate of inflammatory cells in the contralateral joint.

Neuropeptides are known to have an important role in the inflammatory process (7 and 8). They can share and interact with the neuropeptide's second messengers and so can connect certain immunological stimuli to the biosynthesis of inflammatory mediators (7). In addition there has been a report describing the contralateral effect of monoarthritis on the spinal cord (4), but to date little work has been done on the effect of ipsilateral arthritis on the vascular response of the contralateral joint to tachykinins.To further investigate this matter, the experiments described in this section were carried out.

MATERIALS AND METHODS

Experiments were performed on two groups of rats, an intact group as control and an adjuvant treated group. Freund's adjuvant (Sigma, U.S.A) was injected into the knee joint to induce chronic monoarthritis and the temperature and width of the knee joint measured as an assessment of the inflammatory response at 1, 2 and 3. weeks. In addition, the response of the inflamed knee to Substance (SP)(Cambridge research biochemical) at different doses was examined. In these experiments the contralateral effect of adjuvant induced inflanimation was studied at the third week.

Thirty male Wistar rats from an in-house colony (aged 170 days approx. 330 g in weight) were used in this study. The animals were deeply anaesthetised by Hypnorm (fentanyl citrate 0.3 15 mg/ml and fluanisone 10 mg/ml, Janssen: 0.1 ml/300g i.m.) (Janssen U.K) and diazepam (2.5 rngi'kg,i.p). The width of the knee joint was measured by a digital micrometer (Motoyo

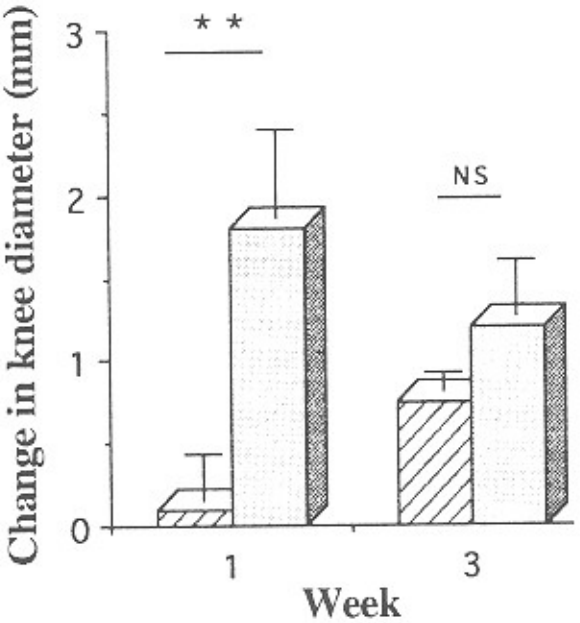

Fig. 1. Changes in knee joint diameter at one and three weeks in response to intra-articular injection of Freun's adjuvant (plain column, n=9) compated to normal (untreated, black column, n=10). ** means differs significantly at week one. P=0.013

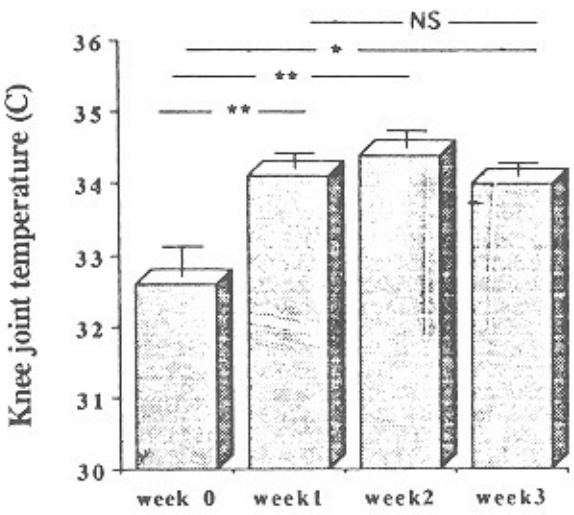

Fig. 3. Changes in contralateral knee joint temperature at three weeks in response to intra-articular injection of Freund's adjuvant compared to week 0. week 0 refers to the experimental starting day. (n=9). * refers to significant difference between week 1, 2 and 3 with week 0. For signficant value see text.

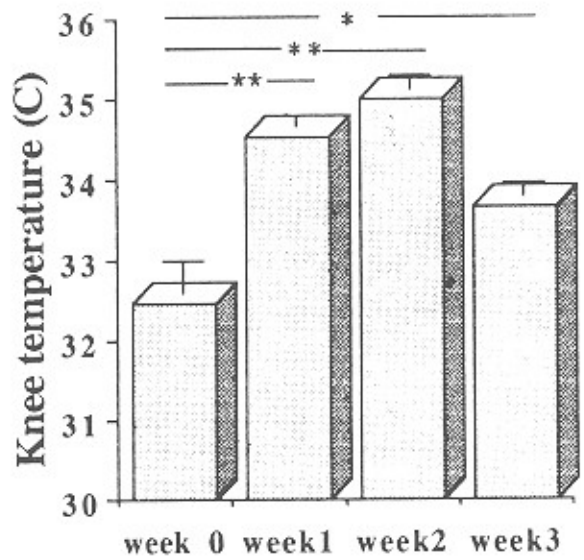

Fig. 2. Changes in joint temperature at three weeks in response to intraarticular injection of Freund's adjuvant. Week 0 refers to the experimental starting day. (control and week 1, n=10, and week 2, n=9, week 3, n=9). * means week 1 differs significantly from week 0. For significant value see text.

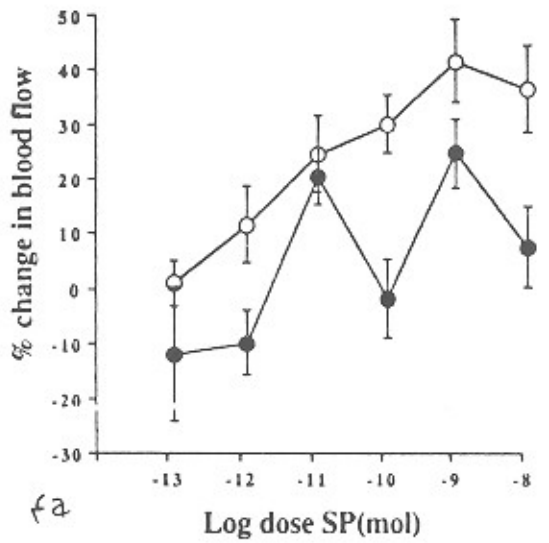

Fig. 4. Changes in synovial blood flow during topical application of SP to the joint capsule in normal. (○, mean ± SEM, n=10), in adjuvant induced chronically inflamed knees at week one (●, mean ± SEM, n=10), and week three (Δ, mean ± SEM, n=8). The sequence of doses administered was randomised.

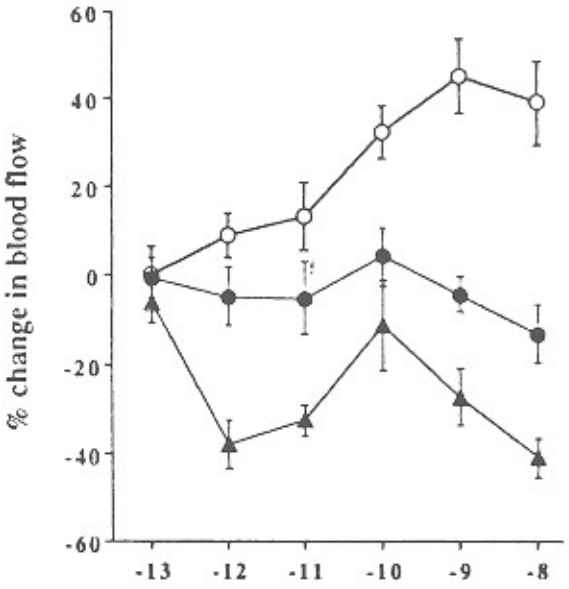

Fig. 5. Changes in synovial blood flow during topical application of SP to the joint capsule in normal. (○, mean ± SEM, n=10), and in contralateral knees at week three of the adjuvant treated animals (●, mean ± SEM, n=9)

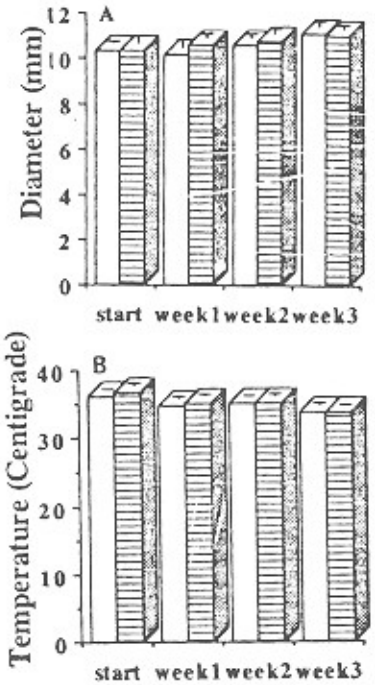

Fig. 6. A and B. Changes in knee joint diameter and temperature at three weeks compared to week 0. Both knees are intact (right knee, doted column left knee lined column). week 0 refers to the experimental starting fay. (n=5). There is not any significant difference between two knee both in diameter and temperature within three weeks.

instruments, Japan) and temperatures were measured by a digital thermometer (Harvard USA). The skin over the knee joint was shaved and 0.2 ml of Freund's complete adjuvant was injected into the synovial cavity of the right knee joint (0.1 ml into the posterior region and 0.1 into the anterior region). The animals were allowed to recover and the inflammatory response was assessed by measurement of knee diameter and temperature at one, two and three weeks post injection. Comparison between mean diameters and temperature was by a two-tail paired student t-test.

For the blood flow experiments, the above animals were deeply anaesthetised by i.p. injection of urethane (2g/kg, Sigma). Here, 10 animals were examined at one and three weeks post injection and a further 10 normal rats were used as controls. An ellipse of skin overlying the joint was excised to expose the medial aspect of the knee. SP (Cambridge Research Biochemicals) was warmed (37 ° C) prior to administration as a bolus applied to the exposed surface of the joint in a volume of 0.1 ml (doses ranged from 10^{-13} to 10^{-8} mol). Warmed (370C) physiological saline (0.9% NaCl) was regularly applied to the knee joint surface to prevent tissue dehydration. Relative charges in synovial blood flow (voltage difference of test minus control which was done by taking a scan before, as control, and after application of drug) were monitored by a laser Doppler perfusion imager (LDI)(Lisca development, Sweden) in both the normal group and the adjuvant treated group. LDI is a modificated laser doppler flowineter which can scan of an area and gives blood distribution data (13). Mean value of results compared with unpaired two tail t student test and Anova with each other and $P < 0.05$ evaluated as significant.

RESULTS

Injection of Freund's adjuvant into the rat knee caused a considerable increase in knee diameter at one week post-injection which got abated by the third week (Fig. 1). At week 1, the adjuvant treated knee width increased by 1.797?0.544 mm (mean ± S.E.M.) compared to week 0 was significantly different ($P=0.013$, n-9, two-tailed paired t-test) from control knees which increased in size due, to the growth of the rats. By week 3, the inflamed knee had only increased by 1.195 ± 0.35 mm from the weĕk 0 level and this was found to be not significantly different ($P=0.3$ 15; n=8 for adjuvant animals and n=10 for untreated animals, two tail t test) from a normal knee which had grown by 0.744 ± 0.11 mm over the three weeks period.

Knee joint temperature also significantly increased at week I in injected compared to control knees and also at week 2 and week 3 the knee temperature in adjuvant treated knee was significantly higher than at

the start (P=0.001, n=9, t test) (Fig. 2).

The contralateral knee did show a significant (P=0.043, n=9) increase in diameter at week one and a significant (p<0.01, n-9, t test) increase in temperature.At week 1 the knee width significantly increased in the contra- lateral knee, compared to week 0 (control) (p=0.043 N=9), but there was no significant difference in diameter of the contralateral knee between week 2 or week 3 compared to week zero. The temperature in the contralateral knee significantly increased at weeks 1,2 and 3 compared to control (week 0) (p<0.05, n=9, t test) (Fig. 3).

Topical application of SP to the control and contralateral knee was performed at week 3. As shown in (fig. 4) the vasodilator effect of SP on the blood flow was significantly attenuated and shifted the dose response curve to the right (p=0.01, n=9, anova).

In the control group temperature and diameter changes over three weeks were not significant and increased knee diameter due to the natural growth at week three was not significant compared to week 0 (p> 0.05, n=5, t test)(fig. 5).

DISCUSSION

The temperature in the contralateral knee was significantly increased at all three weeks compared to control values, (P<0.05, n=9). Knee diameter at week 1 was significantly increased compared to week zero (p=0.01, n=9), but it got abated by week 2 and week 3 and there was not any difference between values obtained at these weeks and the corresponding control values.

SP doses have a vasodilatory effect on blood flow is dose dependent and augmentation of joint blood flow is in dose 10^{-9}Mol which in adjuvant treatment, completely abolished hypereamia and in some instances constrictor response to SP could be observed (fig 4) The attenuation of the effect of SP on blood flow by contralateral inflammation was not as strong on the ipsilateral side but it was significant compared to control. The changes in the temperature and diameter and attenuation of the SP effect on blood flow suggest a neurogenic symmetrical effect. In the control group no significant changes in temperature and diameter of the joint were seen over three weeks. Measurement of ankle diameter and temperature in the ipsilateral and contralateral sides over three weeks did not show any significant (n=15, P> 0.05) changes compared to week zero suggesting that the results cannot be explained by a purely systemic effect of the adjuvant . A functional relationship seems to exist between the sensory nerve fibres in the periphery and neuropeptides in the dorsal horn of the spinal cord. These peptides are therefore well placed to mediate a symmetrical response by a putative central reflex (6). The proposal is that after nociceptor activation secondary to joint damage, preganglionic sympathetic neurones in the autonomic cell column of the spinal cord are selectively activated. These neurones project across the spinal cord to their counterparts on the other side and joint damage could therefore result on both sides (5). Considering these findings and the existence of similar response to SP on the contralateral and at the ipsilateral knees, a neurogenic contralateral effect of adjuvant monoarthritis could be a possible explanation.

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