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β₂- and α₂-Adrenergic Receptor Regulation of TNF Production by Peritoneal Macrophages during Diabetic Neuropathy

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ABSTRACT

The pro-inflammatory cytokine tumor necrosis factor-α (TNF) is a key mediator in development of neuropathic pain. A major source of TNF is macrophages, innate immune cells that express receptors responsive to neurotransmitters. The present work assessed TNF production from macrophages harvested from rats with diabetic neuropathy. Sprague-Dawley rats received intra-peritoneal injection to induce diabetes and were grouped as STZ-induced neuropathic (STZ-DN), STZ non-responder (STZ-NR) and saline-injected (CONTROL). Subsequently rats received bilateral hippocampal injection of either control or small inhibitory RNA (siRNA)-nanoplexes. Peritoneal macrophages were exposed to β₂- or α₂-adrenergic receptor (AR) agonists, lipopolysaccharide (LPS), TNF levels were determined by WEHI bioassay. Our results show for the first time that STZ-DN macrophage with or without LPS stimulation produced greater amounts of TNF and STZ-DN macrophage produced less. Activation of AR on LPS-stimulated macrophage from controls showed opposite response. α₂AR inhibited TNF production, and β₂AR decreased production. α₂AR enhancement of TNF production was not evident in STZ-DN macrophages, and β₂AR inhibition of TNF production was reduced. Increasing TNF activity by siRNA nanoplexes injection reversed the enhanced TNF production by STZ-DN macrophages. Thus, during diabetic neuropathy, there is enhanced macrophage TNF production that may be explained by the altered AR profile.

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BACKGROUND

• Models of neuropathic pain such as chronic constriction injury (CCI) and streptozotocin (STZ)-induced diabetes mellitus (DM) hyperalgesia [1-3] and mechanical allodynia [4] (Fig. 2). Both models increase levels of TNF in regions of the brain involved in pain perception (hippocampus and locus coeruleus (LC)) (Fig. 3) [1,4].

• Tumor necrosis factors (TNF), when administered i.p. to rats, increases the perception of pain [5].

• Increasing TNF in the brain, and only in the hippocampus, can induce neuropathic pain behavior in naive animals [2, 6].

• TNF similar to the activity of an α₂-adrenergic agonist on nerve terminals, inhibits norepinephrine (NE) release from neurons in the hippocampus, a region of the brain rich in adrenergic nerve terminals [7,8].

• Macrophage (Mφ) TNF production is susceptible to regulation by stimulation of the NE-sensitive α₂- and β₂-adrenergic receptors on the macrophages.

-activation of the β₂-adrenergic receptor is pro-inflammatory increasing LPS-stimulated TNF production [9-14].
-activation of the α₂-adrenergic receptor is generally anti-inflammatory inhibiting LPS-stimulated TNF production from Mφs [10,11-13].

• A reversal in peripheral α₂-adrenergic receptor regulation of TNF production from pro- to anti-inflammatory is associated with effective alleviation of CCI-induced thermal hyperalgesia (Fig. 4) [3].

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Supporting Background Data & Methods

Mechanical Allodynia

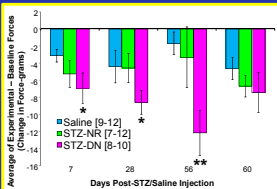


Figure 2. Development of mechanical allodynia following STZ (45 mg/kg, i.p.) injection. Results are presented as the mean ± S.E.M. Statistical analysis by Student's t-test: * p < 0.05, ** p < 0.01, compared to respective saline group. Note: we established ~40% of the STZ-injected rats do not develop consistent allodynia.

Bioactive TNF Levels

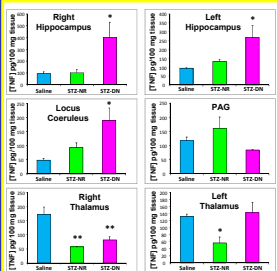


Figure 3. Levels of bioactive TNF in the brain at Day-41 post-STZ/diabetic neuropathy. Data represents the mean ± S.E.M. of three separate experiments. Statistical significance indicated was by ANOVA followed by Fisher's LSD post-hoc analysis. Right Hippocampus, Left Hippocampus and Locus Coeruleus, * p < 0.05, compared to Saline controls and STZ-DN rats; Left Thalamus, ** p < 0.01, compared to Saline controls and STZ-DN rats; ** p < 0.01, compared to Saline controls and STZ-DN rats; ** p < 0.01, compared to Saline controls and STZ-DN rats.

α₂-Adrenergic Receptor Activation in Peritoneal Macrophage

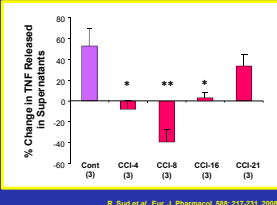


Figure 4. α₂-Adrenergic receptor regulation of TNF production from peritoneal macrophages harvested from rats as indicated on the abscissa. Data are expressed as % change in LPS (30 ng/ml)-stimulated TNF production with controls (10⁶ Mφ) in vitro incubated with treated macrophages stimulated with LPS alone. The value for each group is indicated in parentheses. Statistically significant: * p < 0.05, ** p < 0.01, as compared to the control group. Student's t-test.

Proposed Model for Brain-body Interactions during Neuropathic Pain

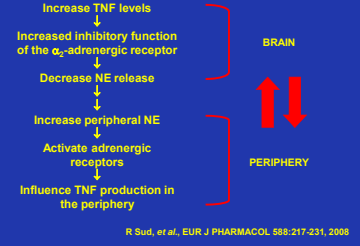


Figure 5. Schematic of proposed changes that occur during development of the central component associated with neuropathic pain and the effect on the peripheral production of TNF by macrophages.

PURPOSE

To determine whether TNF production by peripheral macrophages contributes to the development of neuropathic pain.

Objective: Use a neuropathic pain model distinct from the CCI model to assess TNF production by macrophages.

- STZ-induced diabetic neuropathy
- Determine adrenergic regulation of TNF production by peritoneal macrophages (Figs. 7-9)
- Assess the effect of pain treatment on the production of TNF by macrophages.

METHODS

Male Sprague-Dawley rats, 175-200 grams, were administered a single intraperitoneal injection (45 mg/kg) of streptozotocin (STZ). Rat weights were monitored every other day; blood glucose readings were performed prior to, on day 4 post-STZ, and once a week thereafter for the duration of the study.

Upon sacrifice select brain regions were harvested, processed, and stored until assayed for TNF; levels of TNF were assessed via WEHI bioassay (bioactive protein).

Peritoneal macrophages were harvested by lavage and plated in 100-well Lab-Teks for short-term *in vitro* experiments. Assess the effect of lipopolysaccharides (LPS; 30 ng/ml)-induced TNF production.

Plated peritoneal macrophage were stained to visualize produced TNF, and slides were examined to assess concentration amounts.

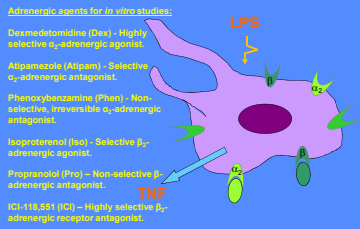


Figure 6. α₂-Adrenergic receptor regulation of TNF production from peritoneal macrophages harvested from rats as indicated on the abscissa. Data are expressed as % change in LPS (30 ng/ml)-stimulated TNF production with controls (10⁶ Mφ) in vitro incubated with treated macrophages stimulated with LPS alone. The value for each group is indicated in parentheses. Statistically significant: * p < 0.05, ** p < 0.01, as compared to the control group. Student's t-test.

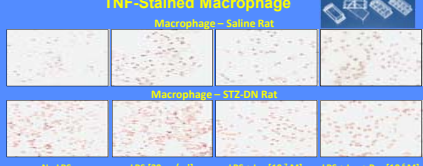
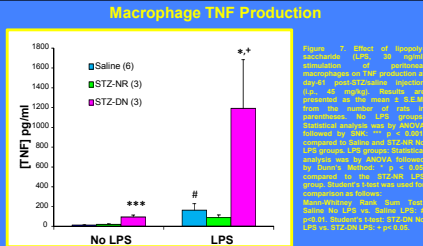
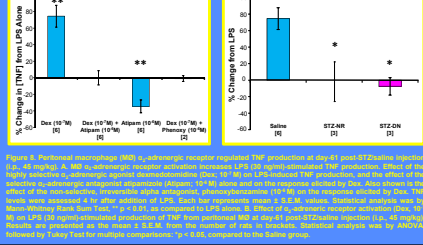


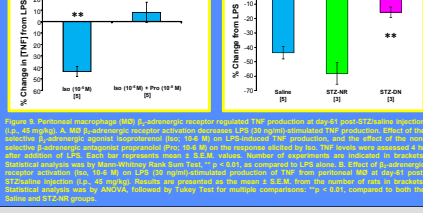
Figure 6. Peritoneal macrophages stained for TNF. Macrophages cultured from diabetic neuropathic control rats (from multiple rats) and STZ-induced rats were incubated with or without LPS (30 ng/ml) for 24 hours. LPS (30 ng/ml) induced TNF production in macrophages from both groups. Representative photomicrographs are shown. Scale bar = 100 μm.



α₂-Adrenergic Receptor Regulated Mφ TNF Production



β₂-Adrenergic Receptor Regulated TNF Production



TNF Nanoplexes Injection (Day-7 post-STZ)

Prevented pain (allodynia) at days-11 and -28 post-STZ. Prevents the increase in hippocampal TNF levels at day-28 post-STZ.

CONCLUSIONS

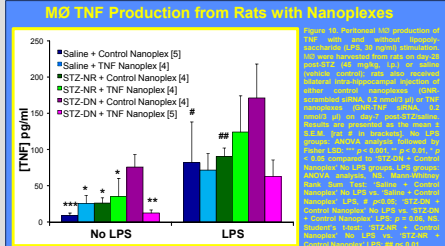
- In rats undergoing development of STZ-induced diabetic neuropathic pain:
 - Peritoneal macrophages from STZ-DN rats are more pro-inflammatory than those from STZ-NR and Saline controls (Fig. 7).
 - A lack of α₂-adrenergic receptor regulation of TNF production from LPS-stimulated macrophages is observed in rats receiving STZ (Fig. 8).
 - β₂-adrenergic receptor regulation of TNF production is less effective in STZ-DN macrophages than in macrophages from STZ-NR and saline control rats (Fig. 9).
 - Inactivation of TNF production in the hippocampus decreases TNF production from rat-LPS-stimulated Mφs and prevents TNF production from STZ-DN rats (Fig. 10).
 - TNF nanoplexes treatment of diabetic neuropathic pain is associated with a return of functional α₂-adrenergic receptor regulation of LPS-stimulated TNF production from STZ-DN Mφs (Fig. 11).
- The differences in adrenergic receptor regulation of LPS-stimulated TNF production by Mφs from STZ-DN and STZ-NR rats may contribute to the lack of neuropathic development in STZ-NR animals.

Intra-Hippocampal Microinjection

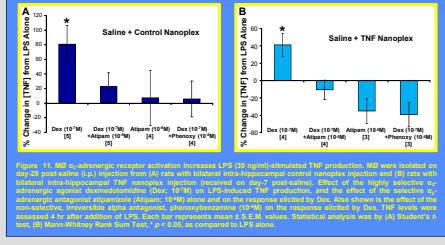
Specifically decreases TNF levels in the hippocampus during chronic pain:



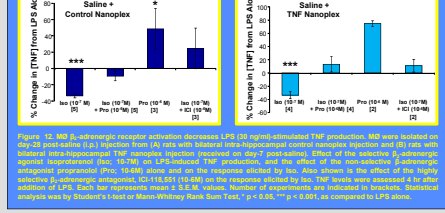
Gold nanoparticles were used to protect and deliver either small inhibitory RNA targeting TNF (TNF siRNA) or scrambled siRNA (control), termed nanoplexes.



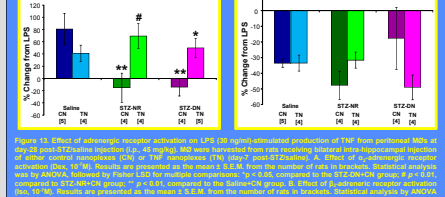
α₂-Adrenergic Receptor Regulated Mφ TNF Production from Nanoplex Injected Rats



β₂-Adrenergic Receptor Regulated Mφ TNF Production from Nanoplex Injected Rats



α₂ and β₂-Adrenergic Receptor Regulated Mφ TNF Production from Nanoplex Injected Rats



Interrelationship of Topics Under Study

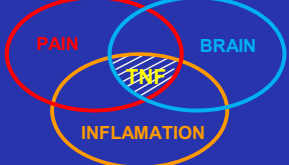


Figure 4. A link common to chronic pain, inflammation and the brain. The relationship between chronic pain and inflammation, while complex, shares similar pathologic changes, including changes in the brain. The interrelationship of the pro-inflammatory cytokines and neural mediator TNF is common to both disorders.