THE EFFECT OF ACUTE SOMATIC PAIN ON THE KILLING ACTIVITY OF NEUTROPHILS IN NEWBORN RATS

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The immune system is subject to all sorts of influences. Pain is one of them, accompanying an organism’s existence. It is essential to be aware of and account for age-related characteristics of the innate immunity in order to adequately assess their dynamics in ontogenesis. The literature is scarce on the changes to the killing activity of neutrophils occurring in newborns in response to acute pain. The aim of this study was to detect potential changes to the phagocytic activity of neutrophils in response to an algogenic stimulus in newborn rats. The experiments were carried out in 3-5-day-old rats. Two groups were formed: the control group and the main group, in which acute pain was modelled. Blood samples were collected 2, 30–60 and 120–180 minutes after exposure to the algogenic stimulus. The microbicidal activity of neutrophils was measured using a spectrophotometric modification of the spontaneous/stimulated nitroblue tetrazolium (NBT) reduction test. The results were compared using the Mann-Whitney U test. In the first hour following pain modeling, the stimulated NBT reduction test demonstrated an increase in the measured parameters from 71.5 to 87.4 a.u. (p < 0.001); the spontaneous NBT reduction test showed an increase from 50.7 to 58.6 a.u. (p < 0.01) 30 to 60 min after exposure. The most pronounced change of the microbicidal activity coefficient was observed 2 min after pain modeling, increasing from 1.40 to 1.72 a.u (p < 0.001). By the end of the experiment, the measured parameters approximated their initial values. During the analysis, we accounted for the fact that the neutrophil response to the algogenic stimulus was unfolding in the setting of microbial colonization occurring in newborns.

Keywords: pain, NBT test, newborns, neutrophils, neutrophil microbicidal activity

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Dynamics of the spontaneous NBT reduction test

Fig. 1. Dynamics of the spontaneous NBT reduction test in newborn rats before and after exposure to the algogenic stimulus. ** — differences are significant between the main group and the control animals (p ≤ 0.05)
57.1 a.u. In the main group, Me\textsubscript{sp NBT} reached 51.4 a.u. \[Q_{0.25} = 48.8 \text{ a.u.}; Q_{0.75} = 54.1 \text{ a.u.}\], Min was 41.9 a.u. and Max was 59.1 a.u. 2 minutes after the algogenic stimulus was applied. In the first hour after exposure, Me\textsubscript{st NBT} was 58.6 a.u. \[Q_{0.25} = 57.0 \text{ a.u.}; Q_{0.75} = 60.5 \text{ a.u.}\], Min was 52.1 a.u. and Max was 61.3 a.u. In the third hour of observation, Me\textsubscript{st NBT} equaled 46.9 a.u. \[Q_{0.25} = 43.3 \text{ a.u.}; Q_{0.75} = 49.8 \text{ a.u.}\], Min was 41.6 a.u. and Max was 51.1 a.u.

Statistical analysis revealed that algogenic stimulation was activating spontaneous NBT reduction. The differences were significant between the control and the main group and also between the initial response to electrical stimulation and the response recorded 30–60 min after it \(p < 0.01\). Median values yielded by the experiment demonstrate that distribution between the upper and lower quantiles was even; the upper quantile approximated the maximum peak value in the sample. It should be noted that the observed reaction was short-lasting and quickly depletable. Two hours after pain modeling, the median values of the spontaneous NBT reduction test were lower than in the control group \(p = 0.05\) (Fig. 1).

The stimulated NBT reduction test produced the following results in the control group: Me\textsubscript{st NBT} = 71.5 a.u. \[Q_{0.25} = 68.0 \text{ a.u.}; Q_{0.75} = 73.4 \text{ a.u.}\], Min = 67.2 a.u., Max = 76.5 a.u. These values were higher than in the spontaneous reduction test.

Immediately after algogenic stimulation, all parameters of the stimulated NBT reduction test started to grow, indicating that neutrophils were highly primed to ward off a potential microbial attack. Specifically, Me\textsubscript{st NBT} was 90.4 a.u. \(p < 0.001\) \[Q_{0.25} = 87.8 \text{ a.u.}; Q_{0.75} = 93.0 \text{ a.u.}\], Min was 84.8 a.u. and Max was 96.3 a.u. Neutrophils retained a high level of microbicidal activity for an hour following exposure to the stimulus: Me\textsubscript{st NBT} = 87.4 a.u. \(p < 0.001\) \[Q_{0.25} = 78.8 \text{ a.u.}; Q_{0.75} = 89.9 \text{ a.u.}\], Min = 77.6 a.u. and Max = 93.0 a.u.

Fig. 2. Dynamics of the stimulated NBT reduction test in newborn rats before and after exposure to the algogenic stimulus. ** — differences are significant between the main group and the control animals \(p \leq 0.05\)

Fig. 3. Dynamics of the microbicidal activity coefficient in newborn rats before and after exposure to the algogenic stimulus. ** — differences are significant between the main group and the control animals \(p \leq 0.05\)
Similar to the spontaneous NBT reduction test, 2 hours after pain modeling microbicidal activity reserves were depleted. Me$_{\text{NBT}}$ declined to 64.5 a.u. ($p < 0.05$) [Q$_{0.15} = 57.2$ a.u.; Q$_{0.75} = 66.9$ a.u.], Min decreased to 54.8 a.u. and Max was 74.1 a.u. (Fig. 2).

Knowing that the modified NBT test reflects the metabolic activity of neutrophils, specifically the oxygen-dependent mechanisms underlying their microbicidal effect, we should consider the opinion of its developers about the killing effect of neutrophils being best described by the microbial activity coefficient [12]; the coefficient is calculated by dividing the value of the stimulated NBT reduction test by the value of the spontaneous NBT reduction test. Me$_{\text{MAC}}$ = 1.33 a.u.; Q$_{0.15} = 1.45$ a.u., Min = 1.26 a.u., Max = 1.47 a.u.

Two minutes after algogenic stimulation, MAC was significantly increased relative to the control value: Me$_{\text{MAC}}$ = 1.72 a.u. ($p < 0.001$) [Q$_{0.15} = 1.68$ a.u.; Q$_{0.75} = 1.80$ a.u.], Min = 1.63 a.u., Max = 2.19 a.u.

In the first hour after exposure, MAC was declining but was still above the values demonstrated by the control group: Me$_{\text{MAC}}$ = 1.49 a.u. [Q$_{0.15} = 1.44$ a.u.; Q$_{0.75} = 1.51$ a.u.], Min = 1.32 a.u., Max = 1.53 a.u.

By the end of the experiment, MAC did not differ significantly from the control values: Me$_{\text{MAC}}$ = 1.34 a.u. [Q$_{0.15} = 1.30$ a.u.; Q$_{0.75} = 1.41$ a.u.], Min = 1.28 a.u., Max = 1.47 a.u. (Fig. 3).

**DISCUSSION**

A theoretical framework for understanding the impact of acute somatic pain on the phagocytic activity of neutrophils can only be developed in the context of neuro-immuno-endocrine interactions. Today, there is no doubt that neurogenic, endocrine and neuroendocrine mechanisms all contribute to maintaining the body’s homeostasis. The pathogenesis of pain or at least its initial stage is similar to the unfolding of stress. Stress is not always caused by pain but acute pain is always a stress, which is why stress hormones, specifically catecholamines, are synthesized in response to an allogenic stimulus. The model used in this study was previously exploited to demonstrate that 2 min after applying an allogenic stimulus, adrenalin and noradrenaline levels, whose ratio is age-dependent, increased in peripheral blood [12].

In another study, the electrophysiological analysis revealed that excitation of nociceptors and signal transmission via the ascending pathways led to the activation of brain structures involved in the control of involuntary functions, the hypothalamus in the first place, and synchronized with the activation of neutrophil granulocytes [13].

Apart from neurogenic noradrenaline, the adrenal medulla releases adrenaline and noradrenaline into the bloodstream. These hormones ultimately find their targets and the subsequent events follow a typical stress scenario. Neutrophils are one of such targets; they were shown express both α- and β-adrenergic receptors [14], which was later confirmed by another study [15]. In our experiments, we observed an increase in the phagocytic activity by the end of the first hour following exposure to the stimulus (in the spontaneous NBT reduction test); in the stimulated NBT reduction test, the phagocytic activity started to increase by minute 2 following pain induction. But by the end of the experiment the phagocytic activity had been restored to the initial level. This can be explained by the activation of beta-adrenoceptors of neutrophils [16]. Also, it is important to remember that stress is accompanied by elevated production of glucocorticoids that inhibit the functional activity of neutrophils [17].

The obtained MAC values suggest that neutrophils of newborn rats can exert killing activity [11, 18]. We have shown that algogenic stimulation leads to both spontaneous and stimulated NBT reduction, as well as to an increase in the killing activity of neutrophils, which, on the surface, seems to be inconsistent with the conventional concept of functional immaturity of phagocytes in newborns.

One of possible explanations for our findings might lie in the acknowledgement of the early formed capacity of neutrophils to produce a superoxide [19] when their protective potential is reduced [20].

In the neonatal period, the organism of the newborn, including skin, mucosal lining, gastrointestinal and genitourinary tracts and lungs, is actively colonized by microbiota. This process is short-lasting and intense. Although this fact has long entered textbooks, it is still explored in the academic literature from different perspectives [21–23]. But one opinion that is held firm is that an intense antigen load cannot but mobilize the factors of immune defense, still allowing for the fact that the mechanisms of adaptive immunity are immature at the time the child is born. One should agree that innate immunity factors in general and neutrophils in particular (as the most labile cells) play a key role in the first-line defense in the neonatal period. The discovery of the bacterial translocation corroborates this hypothesis [24].

Thus, on the one hand, the innate immunity of newborns is stimulated by microbial colonization and, on the other hand, acts as a background for the unfolding response to a different non-antigen stimulus, which in our case was an allogenic stimulus.

**CONCLUSIONS**

1. The killing activity of neutrophils is initiated and enhanced in response to an acute allogenic stimulus. 2. In newborn rats, an antimicrobial response to short-term acute pain demonstrated by neutrophils is short-lived and quickly depletable. 3. The results of this study broaden our knowledge of metabolic activity of neutrophils in response to acute pain in newborns. Our findings can be used in pain research, for adequate assessment of changes occurring in ontogenesis and for prevention of adverse effects pain can have.

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